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Stimulation of the muscles of mastication. *P. J. Brekhus, W. D. Armstrong,* and *W. J. Simon* (University of Minnesota)..... 87

A statistical comparison of cultured and non-cultured root canal cases. *Maurice Buchbinder* (Columbia University)..... 93

Observations on induced dental caries in rats. I. Reduction by fluorides and iodoacetic acid. *F. J. McClure* and *Francis A. Arnold, Jr.* (National Institute of Health)..... 97

Saliva and enamel decalcification. VI. Predisposing caries factors. *J. T. Gore* (Philadelphia, Pa.)..... 107

Amount of organic matter in enamel from several types of human teeth. *Martin Deakins* and *J. F. Volker* (University of Rochester)..... 117

Comparative measurements of salivary pH. *George D. Wessinger* (Loyola University, Chicago)..... 123

Effect of storage temperature on the precipitable protein content and catalase activity of whole saliva. *Martin Deakins* (University of Rochester)..... 129

The incidence of root resorption of vital permanent teeth. *Samuel Hemley* (Columbia University)..... 133

Sulfonamide chemotherapy of dental infections. *Leo Stern* (Mt. Sinai Hospital, New York)..... 143

International Association for Dental Research. Scientific proceedings of the New York section. *Frances Krasnow* (New York)..... 157

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STIMULATION OF THE MUSCLES OF MASTICATION[1]

P. J. BREKHUS, W. D. ARMSTRONG, AND W. J. SIMON

University of Minnesota, Minneapolis, Minn.

Frequent examples of the development of living tissues in accordance with what appear to be functional demands are evident. Tissues that are not used often degenerate, and tissues or organs that are subjected to much use often become strikingly developed. In this fact may lie a hint for future investigators in the field of dentistry.

THE MUSCLES OF MASTICATION

From time to time investigations on the strength of the muscles of mastication have been conducted by various investigators during different periods and in different geographical centers. The earliest study on record, as far as we know, was made in 1681 by G. A. Borelli (1). He records a bite strength as high as 200 kilograms (Ca. 430 pounds). G. V. Black (2) reported in 1895 that he had tested the strength of bite of several thousand individuals and found an average of 175 pounds. L. H. Waugh (3) has now reported the strength of bite among Eskimos and has recorded up to 330 pounds bite pressure. In an experiment made at the University of Minnesota (4) among Minnesota athletes the average strength of bite was found to be 126 pounds pressure, which was the same as the average of the general student body at that time. From the figures available from these studies it is difficult to arrive at accurate statistical conclusions, but it seems that, with the increasing prevalence in dental caries, malocclusions, and gingival diseases, there has also been a decrease in the tone and strength of the muscles of mastication, an observation which is generally accepted. A. H. Thompson (5) in 1876 in a discussion on the dynamics of dental occlusion and the structural expenditure of

[1] Read at the 16th General Meeting of the International Association for Dental Research, Minneapolis, March 12–13, 1938 (*J. D. Res.* **17**: 292. 1938) (Received for publication Dec. 7, 1940).

their maintenance writes: "Suspension of the irritation of use to the parts brings a marked atrophy of bones and muscles, from the withdrawal of nutritive supply and consequent reduction. In age, disease usually renders the teeth, when remaining, unable to bear forcible occlusion, and in this way disuse accelerates the ordinary atrophy of senility."

In the experiment reported below, we are not studying senile atrophy, but the effects on the muscles of mastication in young subjects by supplying additional exercise to the jaws, beyond the ordinary functional demands for masticating modern foods.

EXPERIMENTAL

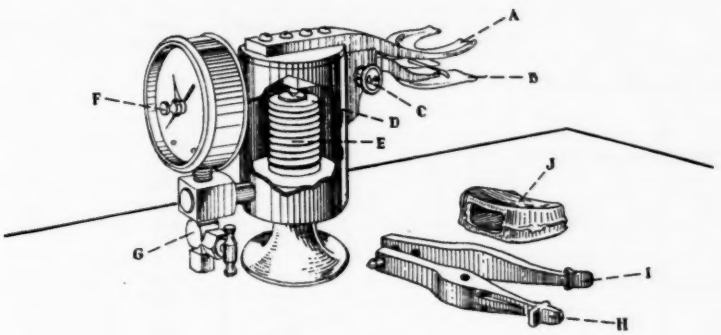
Two classes of male students at the University of Minnesota, School of Dentistry, were chosen as subjects for this experiment. At the outset of the study the strength of bite in pounds pressure of each member in both groups was recorded, the individuals being tested in random order. The hydrostatic gnathodynamometer, *fig. 1*, designed at the University of Minnesota, School of Dentistry, was the instrument used in obtaining these records.[2]

Full cognizance was taken of the fact that an individual rarely registers the same bite pressure trial after trial. This variation in bite pressure of an individual at several trials has been attributed to physiological and psychological factors. To eliminate the physiological practice and fatigue effect in the bite pressures of an individual subject, only the highest registered biting pressure of the subject at each trial period has been used in computing the average bite of the groups. To this end each subject was required to register 3 successive bite pressures at approximately one half minute intervals at each trial; irrespective of the succession, the highest registered bite pressure was selected as "the bite pressure" of the trial.

At the beginning of the experiment the average bite pressure of group 1, composed of 62 male subjects, was 135 pounds. At the close of the study, 9 weeks later, the average bite pressure of group 1 was 136 pounds. This group was not subjected to any experimental procedure.

[2] This instrument was calibrated and approved for mechanical accuracy by the Bureau of Standards, Washington, D. C., in 1937.

The average bite pressure of group 2, composed of 46 male students, was 118 pounds at the beginning of the experiment; this will be designated as the "original bite" of this group. The subjects were given an assignment to chew 1 one-half inch cube of paraffin 1 hour each day for 50 consecutive days.[3] After a number of experiments with several materials, paraffin was selected for its toughness, elasticity, resistance to pressure, and lasting quality. It can be chewed all day without disintegrating and softening in the saliva. Its melting point is 140°F. Sufficient quantity to last for 50 days was issued to each subject at the beginning of the experiment.



A. Fixed biteplate } for all teeth
B. Movable biteplate }
C. Fulcrum
D. Pressure point
E. Siphon
F. Pressure gage
G. Needle valve
H. Movable biteplate } for single tooth
I. Fixed biteplate }
J. Rubber pad

FIG. 1. Hydrostatic gnathodynamometer

At the close of the third week a check bite was taken of the subjects in group 2 who were meeting their assignments. The average bite pressure at the close of the third week was 140 pounds.

[3] Seventy-one men started in this group, but only 46 subjects completed the assignment. The reasons for dropping 25 men were various. Several developed third molar complications during the course of the experiment. Two complained of headaches from chewing paraffin. One complained that the paraffin upset his stomach. Six men wore prosthetic appliances, and they were excused from further experimentation. Three men were having bridgework constructed, and they also were excused. Several men were so negligent of their records that they had to be dropped. All habitual gum chewers were also excluded.

After 50 consecutive days another bite registration was made, and the average was found to be 142 pounds. The results indicated that group 2 had increased the average bite pressure 24 pounds. The question immediately arose as to whether or not this increase was due to practice on the gnathodynamometer. Therefore, two weeks after the completion of the chewing assignment a final bite was taken of group 2, and the average bite was back to 119 pounds, which is almost the same as the original bite.

TABLE I

Statistics of bite pressure in pounds for the 3 groups of subjects

GROUP	NUMBER	ELAPSED TIME IN DAYS*	GROUP AVERAGE	GROUP STAND. DEVIATION
I Males not exercised	62	0	135	28
		50	136	23
II Males exercised 50 days	46	0	118	27
		21	140	28
		50	142	29
		50 + 14	119	24
III Females exercised 50 days	20	0	79	17
		14	86	17
		28	94	18
		50	99	24
		50 + 14	83	21

* That is, from day of "original bite" determination which initiated the observational period.

The experiment was repeated with 20 female subjects, group 3, and it was found to be possible to increase the strength of bite in women also by an assignment of mandibular exercise. The comparative results of this experiment are tabulated in *fig. 2*.

In considering the differences that occur (Table I and *fig. 2*) it is important to note that all bite recordings were made by the same operator using the same instrument (graduated to 5 pound increments). Whether a male subject belonged to group 1 or group 2 was not known to the operator, nor was he aware of any individual's bite pressure recorded at any previous test.

The "original bite" average readings differ significantly from one

group to another. For this we have no satisfactory explanation, although it is relevant to note that the groups originated from three different university classes, the junior and senior dental students and the freshman dental hygienists. This significant differentiation in the original bite of the three groups is not of serious consequence to our study, however, as our interest is centered in the average changes within the groups as the experiment proceeded.

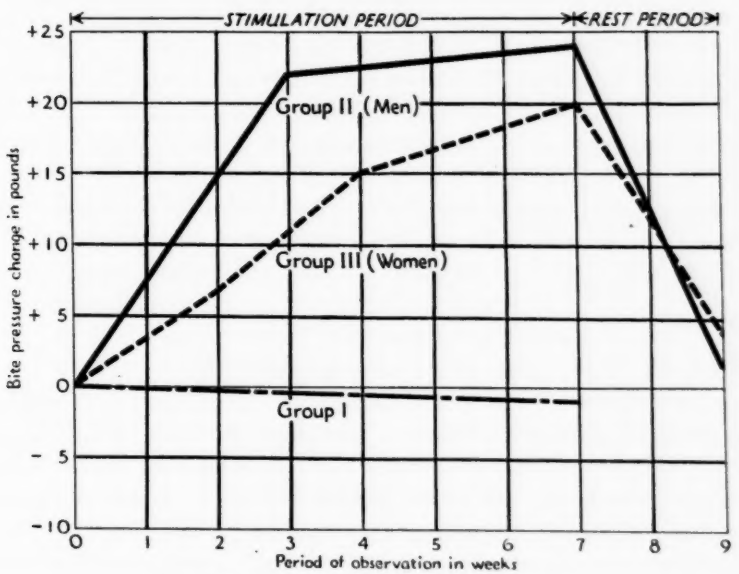


FIG. 2. Comparative results of muscle stimulation

CONCLUSIONS

The above experiment seems to indicate that the strength of the muscles of mastication is influenced by the amount of use to which they are put, up to a physiological limit. In this respect they do not appear to differ from muscles in any other part of the body. What significance this has for the soft tissues of the oral cavity, the development of the jaw bones, or the health of the dental structures is not known, but the present experiment indicates the usefulness of further studies along this line.

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A STATISTICAL COMPARISON OF CULTURED AND NON-CULTURED ROOT CANAL CASES[1]

MAURICE BUCHBINDER, B.S., D.D.S.

School of Dental and Oral Surgery, Columbia University, New York, N. Y.

The inherent advantages in the culturing of root canals are many and have been stressed by root canal workers in the past. They are largely of a "common sense" nature. There is an obvious check on gross contamination of the field of operation as well as the instruments employed. By cultures the dentist is able to detect the presence of live organisms, regardless of the production of odor. Furthermore the root canal worker is thereby enabled to compare the relative value of two different methods of treatment, using for a yardstick the standard of periapical sterility as determined by cultures.

The purely "common sense" justification for the culturing technic is not, however, entirely satisfying. Since root canal culturing technic has been simplified by the use of standard prepared media, such as Difco, tubed in screw cap vials (5), it would be desirable to prove a scientific value for culturing. Not much light has been shed on the critical question as to whether a tooth which has been treated under control of the culturing technic stands a better chance of healing than one which has not. Several writers have reported on a statistical basis the results obtained in the treatment of pulpless teeth. These include Coolidge (1), Auerbach (2), and Buchbinder (3). Recently, Yates and Morse (4) reported large groups of cases. None of these studies compares a group of teeth which has been cultured, against a group which has not been cultured, all other factors remaining equal.

The present report is concerned with such a comparison. All teeth were treated in the root canal clinic of Columbia University by senior students working under close supervision between 1930 and 1940.

[1] Read at a meeting of the Root Canal Study Club of Philadelphia, May 15, 1940. (Received for publication, Nov. 21, 1940.)

METHOD

During the entire period, the teeth were treated in a uniform manner: Asepsis by rubber dam, autoclave sterilization of dressings, chemical sterilization of instruments. Medication consisted of Dakin's solution for irrigation of the canal, creosote-iodin and eugenol for the dressings. In selecting cases for treatment, known focal diseases were excluded, and obvious cystic conditions were ruled out by radiograph. Beyond this there was no exclusion, regardless of size of the radiographic lesion, or of the presence of fistula, swelling or pain. Nearly all the teeth were single rooted.

The first group of cases which included those teeth treated between 1930 and 1936 were filled on the basis of a clinical criterion. That is, they were filled when locally comfortable, when the seepage appeared clean and not excessive and when the dressing seemed clean. For the second group of cases, those between 1936 and 1940, the culturing test was introduced. The medium was first dextrose broth, but was soon changed to brain heart infusion. At first a single negative culture was required but this was later changed to 2 negative cultures. The filling method was the same for all teeth and has been described elsewhere (3).

All cases were recalled every 6 months for radiographic and clinical check for as long a time as the particular patient cooperated. It is on the data furnished by these check-ups that the report is based.

DATA

A total of 245 teeth are included in the report. All were diagnosed originally as having necrotic pulps, with radiographic evidence of involvement.

At present each tooth is classified as Normal, Improved or Failure. These terms are defined as follows: *Normal*—Original radiographic abnormality has been completely restored to normal, at the time of the last check-up; *Improved*—Original radiographic abnormality has been decreased in size, but cannot as yet be considered normal; *Failure*—Original radiographic abnormality is either static or worse (larger in size).

DISCUSSION

In the statistical treatment of clinical data there are several basic principals. First, the cases in the 2 groups which are being compared should be quite similar to each other as a whole, except for a single variable. In the present study this condition has been approximated, since the method of selecting cases, the type of treatment, and other relevant factors have all been kept constant, excepting for the single point that in one group the culturing criterion was used and in the other it was not.

Whether any particular root canal infection will heal obviously

depends on a complex of forces, some of them not measurable, i.e., tissue resistance and virulence of the infecting organism. It is another basic principal of statistical analysis, that in comparing large and similar groups, all these factors will distribute themselves evenly, and

TABLE I

Results of treatment of 151 cultured and 94 non-cultured cases

		AVERAGE TIME OF FOLLOW-UP
CULTURED GROUP:		
Total Number	151	
Normal	110 (73 per cent)	17.5 months
Improved	30 (19 per cent)	9 months
Failure	11 (8 per cent)	
NON-CULTURED GROUP:		
Total Number	94	
Normal	59 (63 per cent)	21.8 months
Improved	18 (19 per cent)	13.8 months
Failure	17 (18 per cent)	

TABLE II

Statistical analysis of 121 cultured and 76 non-cultured cases

CULTURED GROUP:			
Total Number of Cases	121		
Normal	110	Proportion of Normal	.909
Failure	11	Proportion of Failure	.091
		Standard Error of Normal*	.025
NON-CULTURED GROUP:			
Total Number of Cases	76		
Normal	59	Proportion of Normal	.776
Failure	17	Proportion of Failure	.224
		Standard Error of Normal*	.046

* Standard Error of Difference between proportions of normal in cultured and non-cultured groups, or Sigma (calculated as the root mean square of the standard error of each proportion) = .053; Actual difference (.909 − .776) = .133

will cancel each other out in any comparison. Consequently the effect created by any one variable will become clearly visible.

Finally, large enough groups of cases must be used so that any difference detected between the two groups will be statistically signifi-

cant. The root mean square deviation method was used in the calculations.

There are two main groups being compared, the cultured group of 151 cases and the non-cultured group of 94 cases (Table I). The cultured group yielded 8 per cent failure, while the non-cultured group yielded 18 per cent failure. By calculating the deviation of the normal in each group, one can determine if the observed difference in the behaviour of the two groups is significant.

A question arises as to what to do with the improved cases in each group. This, it must be remembered is a transient class and at later examinations a tooth marked Improved may become either Normal or Failure. The simplest way is to omit such cases, reducing the number of cases for each group. Deviations were calculated on this basis (Table II). Similar results were obtained, however, by absorbing the Improved cases into the Normal and Failure classes, according to the proportions existing in each of the two main groups.

If the observed difference between the proportions is more than twice the standard error of the difference, then the difference is considered to be significant.

SUMMARY

Two groups of root canal cases were followed up by periodic examination after treatment. In one group the culturing criterion was used and in the other it was not. Statistical analysis reveals that significantly better results were obtained when culturing was used.

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OBSERVATIONS ON INDUCED DENTAL CARIES IN RATS

I. Reduction by Fluorides and Iodoacetic Acid

F. J. McCLURE, M.S., Ph.D.[1] and FRANCIS A. ARNOLD, Jr., B.S., D.D.S.[2]

National Institute of Health, U. S. Public Health Service, Washington, D. C.

The communal water supply at Galesburg, Illinois, as compared with that of Quincy, Illinois, was shown to have a marked beneficial effect in inhibiting human dental caries (1). This finding based on epidemiological evidence was tested experimentally by studying the effects of these two drinking waters on induced caries in rats. The results obtained thus far have proved inadequate to warrant definite conclusions, but the data is none the less useful to call attention to what may be an important factor in the problem of induced caries in rats, namely, a possible litter association as to caries susceptibility existing among experimental rats.

Other data in this paper, which is the first of a series of papers reporting some recent observations on rat caries made at the National Institute of Health, relate to the effect of: (a) 2 ppm.[3] of fluorine as sodium fluoride in the drinking water; (b) 125 ppm. of fluorine as sodium fluoride in the drinking water; (c) 125 ppm. of fluorine as sodium fluoride in the ration, and (d) 200 ppm. of iodoacetic acid in the ration, together with 20 ppm. of iodoacetic acid in the drinking water.

Miller (2) reported the prevention of induced caries in rats by feeding fluorides and iodoacetic acid. His results with respect to fluorides were confirmed by Finn and Hodge (3). Cox, Matuschak, Dixon, Dodds and Walker (4) sought to show a reduced incidence of caries in rats due to fluorides contributed to the offspring by mother rats during gestation and lactation.

The first production of carious-like lesions in rats molar teeth is

[1] Associate Pharmacologist, Division of Infectious Diseases. (Received for publication January 29, 1941.)

[2] Passed Assistant Dental Surgeon, Division of Infectious Diseases.

[3] ppm. = parts per million.

attributed to Shibita (5) who used a diet containing unground rice and greens mixed with various sugars. Coarse ground corn meal as contained in the Hoppert, Webber and Canniff diet (6) will produce similar lesions. Rosebury, Karshan and Foley (7) have studied the etiology of this type of rat caries and have collected much data with respect to dietary variations including minerals, vitamins, fats, carbohydrates, and proteins as factors affecting the rats response to a caries-producing diet.

In the studies reported in this paper and in the succeeding papers to follow, the simplified Hoppert corn meal ration as used by Cox and his co-workers was fed, and the system of recording and scoring occlusal rat caries as proposed by Cox, Dodds, Dixon and Matuschak (8) was followed.

EXPERIMENTAL

The effects of Galesburg and Quincy drinking waters and 2 ppm. fluorine water were tested on litters of rats whose mothers had also received these waters prior to and during gestation and lactation. The experiment included a control group given distilled water. All the breeding females were given a stock ration. The young were separated at 21–23 days of age and were fed in groups according to sex. They were given a caries-producing ration consisting of 66 per cent granulated corn meal (approximately 60 per cent of this meal was retained by a 40-mesh sieve), 30 per cent whole milk powder, 3 per cent alfalfa meal (all of which passed a 60-mesh sieve utilized to remove particles of sand which may contaminate alfalfa meal) and 1 per cent sodium chloride. The drinking waters of these 4 groups were the same as received by their mothers. Another group was given 125 ppm. fluorine in the drinking water but no added fluorine was given to the parents of these rats. Initial and final weights were determined for each rat. At the end of 15 weeks the rats were killed, the heads and leg bones cleaned of all adhering tissue and dried. The molar teeth were examined under a low power microscope (magnification × 22.5), the visible cavities being recorded and scored on a chart, described and illustrated in the article by Cox, Dodds, Dixon and Matuschak (8). Only the cavities as seen in unsectioned teeth were recorded. The examination and scoring of each set of teeth was checked by both authors.

The experimental plan to test the effects of 125 ppm. of fluorine in the ration, and iodoacetic acid in the ration and drinking water, did not involve any prior treatment of the breeding stock. Young stock rats were taken at 21 days of age and litter mates were divided among 3 groups, i.e., control group, one fed fluorides, and the other given iodoacetic acid. Each litter of rats was represented in each group by 1 or 2 litter mates. No distinction was made as to sex, which does not appear to be a factor influencing rat caries.

The rations for this experiment consisted of the basal ration described above with certain additions. Fluoride (125 ppm. as NaF) was added to the ration in the form of a dilute solution sprinkled over the corn meal and thoroughly mixed with it. This corn meal was dried in a current of warm air to its original weight. Iodoacetic acid in solution was added to corn meal in a similar manner so that the final ration would contain 200

TABLE I

Effects of Galesburg and Quincy waters and waters containing 2 ppm. and 125 ppm. fluorine as sodium fluoride

LITTER NO.	NO. OF RATS	NO. OF RATS WITH CARIES	TEETH AFFECTED						AREAS AFFECTED						SCORE	
			Upper		Lower		Total		Upper		Lower		Total			
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Total	Ave. per rat
Controls—distilled water group																
3-8-301	6	6	2	5.5	25	69.4	27	37.5	2	3.3	66	45.8	68	33.3	156	26.0
3-7-301	4	2	0	0.0	5	20.8	5	10.4	0	0.0	6	6.2	6	4.4	14	3.5
3-4-303	6	6	3	8.3	25	69.4	28	38.9	4	6.6	93	64.6	97	47.5	275	45.8
3-1-302	6	6	10	27.7	22	61.1	32	44.4	13	21.6	45	31.2	58	28.4	145	24.2
3-2-300	5	5	3	10.0	22	73.3	25	41.7	4	8.0	63	52.5	67	39.4	184	36.8
Quincy water group																
1-3-102	8	8	1	2.1	28	58.3	29	30.2	1	1.2	56	29.2	57	21.0	142	17.8
1-4-103	5	5	2	6.7	16	53.3	18	30.0	2	4.0	26	21.6	28	16.5	60	12.0
1-6-106	8	8	4	8.3	33	68.7	37	38.5	5	6.2	115	59.9	120	44.1	322	40.3
1-7-101	5	3	0	0.0	11	36.7	11	18.3	0	0.0	22	18.3	22	12.9	53	10.6
1-8-101	8	7	0	0.0	32	66.7	32	33.3	0	0.0	72	37.5	72	26.5	180	22.5
1-9-101	8	8	2	4.2	43	89.6	45	46.9	2	2.5	144	75.0	146	53.7	396	49.5
Galesburg water group																
2-12-201	5	2	0	0.0	2	6.7	2	3.3	0	0.0	2	1.6	2	1.2	3	0.6
2-11-201	4	4	1	4.2	14	58.3	15	31.3	2	5.0	30	3.1	32	23.5	71	17.7
2-10-205	6	5	1	2.8	20	55.5	21	29.2	1	1.6	51	35.4	52	25.5	139	23.2
2-9-204	5	3	0	0.0	10	33.3	10	16.7	0	0.0	25	20.8	25	14.7	65	13.0
2-2-202	9	9	17	31.5	39	72.2	56	51.9	22	24.4	138	63.9	160	52.3	432	48.0
2 ppm. fluoride water group																
4-2-401	9	9	0	0.0	25	46.3	25	23.2	0	0.0	54	25.0	54	17.6	130	14.4
4-1-401	6	5	1	2.8	20	55.6	21	29.2	1	1.6	50	34.7	51	25.0	115	19.2
4-3-401	6	6	4	11.1	27	75.0	31	43.1	7	11.7	93	64.6	100	49.0	285	47.5
125 ppm. fluoride water group																
4-5-401	7	3	0	0.0	3	3.6	3	1.8	0	0.0	4	2.4	4	1.7	6	0.9
4-6-401	9	3	0	0.0	4	3.7	4	1.9	0	0.0	4	1.9	4	1.3	7	0.8
2884-A	2	2	1	8.3	3	25.0	4	16.7	1	5.0	3	6.3	4	5.9	4	2.0
2883-A	4	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2870-A	1	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2871-A	3	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

ppm. iodoacetic acid. The corn meal for the control ration was treated with a quantity of distilled water equal to that used for a test ration, and it was similarly dried to its original weight.

The control group and the fluoride group received distilled water for drinking. The iodoacetic group received drinking water containing 20 ppm. of iodoacetic acid. These levels of fluoride and iodoacetic acid are similar to the quantities fed by Miller (2). It was not considered desirable to add yeast to the diet of rats receiving iodoacetic acid, as

TABLE II

Summary of the effects of Galesburg and Quincy waters and waters containing 2 ppm. and 125 ppm. fluorine as sodium fluoride

TREATMENT		NO. OF RATS	NO. OF LITTERS	RATS WITH CARIES	CARIOUS TEETH			AREAS AFFECTED			SCORE* TOTAL
					Upper	Lower	Total	Upper	Lower	Total	
Control (distilled water)	No.	27	5	25	18	99	117	23	273	296	774
	Per cent	—	—	92.6	11.1	61.1	36.1	8.5	42.1	32.2	—
	Ave. rat**	—	—	—	0.7	3.7	4.3	0.9	10.1	11.0	31.0
Quincy water	No.	42	6	39	9	163	172	10	435	445	1153
	Per cent	—	—	92.9	3.6	64.7	34.1	2.4	43.2	31.2	—
	Ave. rat	—	—	—	0.2	3.9	4.1	0.2	10.4	10.6	29.6
Galesburg water	No.	29	5	23	19	85	104	25	246	271	710
	Per cent	—	—	79.3	10.9	48.9	29.9	8.6	35.3	27.5	—
	Ave. rat	—	—	—	0.7	2.9	3.6	0.9	8.5	9.3	30.9
2 ppm. F in water	No.	21	3	20	5	72	77	8	197	205	530
	Per cent	—	—	95.2	4.0	57.1	30.6	3.8	39.1	28.7	—
	Ave. rat	—	—	—	0.2	3.4	3.7	0.4	9.4	9.8	26.5
125 ppm. F in water	No.	26	6	8	1	10	11	1	11	12	17
	Per cent	—	—	30.8	0.6	6.4	3.5	0.4	1.8	1.4	—
	Ave. rat	—	—	—	0.04	0.4	0.4	0.04	0.4	0.4	2.1

* Score averages are based on the number of rats having caries.

** Averages for carious teeth and areas affected are based on the total number of rats in the group.

was done by Miller (2) to obviate a possible toxic action of iodoacetic acid (9). Judging from our growth rates and from gross observations based on all the rats in the control and test groups, this level of iodoacetic acid as fed was innocuous. The rats were kept on the above rations and drinking waters for 15 weeks, when they were killed and the teeth examined as above.

DATA

The amount and degree of caries in the rats may be judged from the data appearing in the accompanying tables, Tables I, II, and III.

As may be seen, the bases for comparison of the various groups of rats are: (a) the number and per cent of rats in each group with caries, i.e., having one or more carious teeth; (b) the number and per cent of carious teeth (molars) per group; (c) the number and per cent of affected tooth areas per group as indicated by Cox's chart,[4] and (d) the average total score of the rats having caries based on Cox's chart which permits an evaluation of the size of each cavity developed in any of the specified areas by giving it a numerical score of 1, 2, or 3.

TABLE III

Effects of sodium fluoride in ration, and iodoacetic acid in ration and drinking water

TREATMENT		NO. OF RATS	NO. OF LITTERS	RATS WITH CARIES	CARIOUS TEETH			AREAS AFFECTED			SCORE* TOTAL
					Upper	Lower	Total	Upper	Lower	Total	
Control	No.	25	14	19	7	67	74	9	125	134	264
	Per cent	—	—	76.0	4.66	44.67	24.66	3.60	20.83	15.76	—
	Ave. rat**	—	—	—	0.28	2.68	2.96	0.36	5.0	5.36	13.89
125 ppm. F as sodium fluoride in ration	No.	25	14	3	1	2	3	1	2	3	4
	Per cent	—	—	12.0	0.66	1.32	1.0	0.40	0.33	0.35	—
	Ave. rat	—	—	—	0.04	0.08	0.12	0.04	0.08	0.12	1.33
Iodoacetic acid (200 ppm. in ration and 20 ppm. in water)	No.	24	12	8	2	17	19	2	20	22	36
	Per cent	—	—	33.3	1.39	11.80	6.60	0.83	3.47	2.70	—
	Ave. rat	—	—	—	0.08	0.71	0.79	0.08	0.83	0.91	4.50

* Score averages are based on the number of rats having caries.

** Averages for carious teeth and areas affected are based on the total number of rats n each group.

Average rat scores based on a litter as a group are also shown in Table I.

RESULTS OF EXPERIMENT

The results which pertain to the Galesburg water, Quincy water, and the 2 ppm. fluoride water groups, and their distilled-water control

[4] There are a total of 34 areas distributed over the 12 molar teeth, in which areas cavity formation is most likely to originate. The upper first and second molars and the lower third molars each present 2 such areas, the upper third molars have 1 area each, and the lower first and second molars have 5 areas each.

group, are the first extensive published data illustrating what appears to be a litter membership factor involved in the production of rat caries [diagnosed more specifically as occlusal caries by Cox, Dodds, Dixon and Matuschak (8)]. For example a somewhat different caries picture is apparent among the 9 rats of litter 2-2-202, Table I, each rat showing from 4–7 affected teeth, as compared with the 5 litter mates of litter 2-12-201, Table I;—only 2 rats of this later litter were carious having but 1 carious tooth each. It thus appears from our limited data that certain variations in rats grouped as to litter membership are truly abnormal as compared with the distribution of the data selected at random. Such results are indicative of a significant finding and the entire question deserves further examination and study. There is no information that a specific caries response is a hereditary characteristic of all the litters of a given male and female mating, or is due to variable conditions which may occur during gestation and lactation or during the actual caries-producing period.

The summary data as shown in Table II relates to all the rats of a group, disregarding the rats litter membership. This does not appear to be the logical treatment of such data which possesses a natural grouping in accordance with prenatal and lactation effects as well as by certain observed litter membership tendencies toward caries. The coefficient of variation for the data where the result per litter is a single item is quite large, and many more litters per group than shown here would be required to demonstrate significant differences on this basis. Thus using the litter average data for rats with or without caries, based on a total of 19 litters (all litters except those given 125 ppm. fluorine), there is obtained a coefficient of variation of 22.5. Referring to the method proposed by Mitchell and Grindley[5] for

[5] University of Illinois, Agricultural Experiment Station, Bulletin No. 165, July 1913. The Element of Uncertainty in the Interpretation of Feeding Experiments by H. H. Mitchell and H. S. Grindley.

These authors' formula is based on the assumption that odds must be at least 30 to 1 that the difference between the means of two groups of data is statistically significant, i.e., the difference should equal roughly three times its probable error. The formula as derived by Mitchell and Grindley is as follows:

$$n = \left[\frac{1.849\ C\sqrt{2 + (\frac{1}{2}c)^2}}{100c}\right]^2$$

C—coefficient of variation (assumed to be equal in both comparative groups)
c—.10 (a 10 per cent difference between averages for each group).

calculating the number of animals that should be included in experimental lots on the basis of the variability of the individual data, it may be shown that roughly 35 litters per group would be required to demonstrate a 10 per cent significant difference between groups made up of litter units.[6]

The results relative to the effects of Galesburg water, Quincy water, and 2 ppm. fluoride water do not show any differences which are statistically significant. The data relative to 125 ppm. of fluorine justify the conclusion that this level of fluorine in the drinking water has significantly inhibited caries development in our experimental rats.

The response of rats given a caries-producing diet to the beneficial effects of 125 ppm. of fluorine in the ration, and to the effects of iodoacetic acid in the ration and drinking water, is strikingly illustrated by the data presented in Table III. The reduction of caries in the fluoride group below that established by their litter mates in the control group is unquestionable, as brought out by all items tabulated to establish the caries picture for the groups. In these groups it is to be noted that each rat in a test group is represented by a litter mate in the control group, and 12–14 litter trios are involved in the study. Differences between the control group and each of the test groups are both more than 100 per cent. There can be no doubt that this is a significant finding. A significant result seems now established as an effect of iodoacetic acid. Both these results are in confirmation of previous results first reported by Miller (2). In comparing this experiment with Miller's report, attention might be called to the fact that yeast was introduced into his iodoacetic acid ration.

It is interesting to note the difference in the rats of the 2 control groups shown in Tables II and III. The control rats represented in Table III, as compared with Table II, were from different breeding strains which may account partly for the different control results.

[6] A discrepancy may reside in other data of this type based on group averages, particularly where the number of rats in a group is small and no data are given as to litter distribution (4, 7). In all due fairness to such group data it must be noted, however, that up until the time the pronounced effects of fluorides and iodoacetic acid on caries were reported (2, 3), group averages generally did not show very significant differences among groups receiving different treatments. This may be an indication that such group averages were obtained with proper recognition of litter membership, but it may also indicate a need for other comparisons being made with more animals, particularly where small differences are presented as representing significant results.

It is to be noted also that the corn meal for the control rats in Table III was treated with distilled water and then dried, which may have modified its caries-producing properties. It is to be remembered also that an uncontrolled difference in fluorine intake from the basal rations fed these 2 control groups could account for some difference in response.

DISCUSSION

In the present state of our knowledge, there are few adequate available data directly suited to explain these observed effects of fluoride and iodoacetic acid on induced rat caries. Laboratory observations such as these have followed the epidemiological studies regarding the association of certain communal water supplies known to contain fluorides, and human dental caries (10). A rôle of fluorine contained in the drinking water is definitely implied in these studies. This current new approach to the study of caries not only has furnished important facts regarding dental caries, but it gives rise to the possibility of partial control of human dental caries.

These observed anti-caries effects of fluorides have at least 2 possible modes of action, either one of which still lacks adequate experimental confirmation. The activity of fluorine as an anti-enzymatic agent lends weight to the belief that fluorine may prevent bacterial degradation of carbohydrates in food deposits. Fluorine, when deposited in the enamel particularly, as shown by Armstrong and Brekhus (11), may, on the other hand, bring about a more acid-resistant, possibly anti-enzymatic tooth material. The effect of a modified saliva and the involvement of more subtle surface phenomena can not be dismissed as possible factors also.

There are certain little understood facts which relate to the production of caries in experimental rats. In the minds of all workers in this field it is unfortunate first, that a coarse, hard particle diet must be used to produce rat caries because it introduces the uncertain element of fracture; secondly, a diet containing a natural ingredient such as whole ground corn leaves something to be desired by way of adequate dietary control. On the other hand, it is not improbable that the efficacy of the coarse particles of corn meal or rice may as well be referred to as a food impaction phenomena. In fact the prevention of rat caries by fluorine in itself may be taken as evidence against the belief that a mechanical factor is the essential feature of these caries-

producing diets. These facts, by assumption at least, relate the etiology of rat caries to that of human dental caries.

SUMMARY

A considerable amount of data relative to the effects of Galesburg (Ill.) drinking water, Quincy (Ill.) drinking water, and water containing 2 ppm. fluorine as sodium fluoride on induced corn meal rat caries has been accumulated. This data has been found inadequate to demonstrate different effects, but it calls attention to a litter membership factor in rats with respect to their caries susceptibility.

The presence of 125 ppm. of fluorine as sodium fluoride in the food or water and the presence of 200 ppm. of iodoacetic acid in the food, together with 20 ppm. of iodoacetic acid in the drinking water, significantly reduce the production of caries in rats fed a caries-producing corn meal diet.

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SALIVA AND ENAMEL DECALCIFICATION

VI. Predisposing Caries Factros[1]

J. T. GORE, D.D.S.

Philadelphia, Pa.

In a previous report (1) an effort was made to show the manner in which the various forms of enamel decalcification take place as the result of the chemical composition of the saliva and the chemical and physical changes which take place in the stagnant saliva.

In that report it was stated that, "The type, degree and rapidity of enamel decalcification depend upon the morphology and chemical composition (ratio of $Ca_3(PO_4)_2/CaCO_3$) of the enamel, the concentration of the $NaHCO_3$ in the saliva (first line of defence), and the ratio of

$$\frac{\text{Potential Ca and } PO_4\text{-ion concentration}}{\text{Potential H-ion concentration}}$$

in the precipitated mucin."

The predisposing factors concerned with the morphology and chemical composition of enamel (calcification), the concentration of $NaHCO_3$ (titratable alkalinity and pH) and the concentration of the $Ca_3(PO_4)_2$ in the saliva have been discussed at length in the literature and are still highly controversial. Excellent summaries of prevailing opinions have been presented by various authors among whom are those of Schour, Karshan, Rabkin, Rosebury and Gordon, including extensive bibliographies, in Gordon's recent book (2).

It is with the predisposing factors influencing the concentration of the potential H ions (carbohydrates) in the saliva that this report is concerned. Clinical observation seems to indicate that susceptibility to dental caries is frequently associated with the excessive consumption of carbohydrates or certain types of carbohydrates (refined). This correlation is not always apparent as there is a tend-

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ency to conceal, either intentionally or unintentionally, the excessive consumption of sugars and starches. Important facts in regard to the diet are often omitted, such as the eating of candy, cakes, cookies, crackers, etc., between meals or before retiring.

There does seem to exist, however, a considerable number of individuals in whom susceptibility or immunity can not be traced to the diet. Digestion and absorption may be a factor in some of these cases, but the most promising fields of investigation are the endocrine glands, especially the pancreas, and other factors involved in Ca and P metabolism.

Mucin is a glycoprotein containing a protein and a carbohydrate radical. Saliva contains considerable quantities of mucin, especially the mandibular (submaxillary and sublingual) saliva. After describing at some length various analytical methods for identifying the carbohydrate in mucin, and the results obtained, Mathews (3) noted "It is clear from the foregoing that the composition of the carbohydrate group in mucin is very uncertain.... There seems to be a non-fermentable sugar in most of these glycoproteins and its nature is still undetermined."

Kirk (4) stated,

> "Certain results have been obtained which are at least significant that the composition of the saliva exerts a most important influence upon the causation of caries and that in connection with the nutritional changes which influence the composition of the mixed oral secretion, we shall in due time discover the true predisposing factor of dental caries. In the examination of a considerable number of specimens of saliva I have found in all cases where true caries were prevalent but especially in the case of children the presence of a dissolved carbohydrate which responded to v. Jaksch's iodine test for glycogen or erythrodextrin. The finding of glycogen in the saliva has been previously reported by Salomon (vid. Maly's Jahresbericht, vii 55), and by Michaels (Trans. iii Congres Dentaire Internat. Paris 1900)."

Later, Kirk (5), in speaking of Michaels' work, said "Among other substances which his methods of analysis have enabled him to detect in the composition of the mixed saliva, was a fermentable carbohydrate substance answering to the tests for glycogen." Elsewhere in the same paper (5) he wrote,

> "My observations on the subject both from the standpoint of laboratory experiment and from clinical observation led me to the conclusion that the saliva of the caries-susceptible is characterized by the presence of this fermentable carbohydrate, which Michaels

has denominated glycogen, and that its absence from the salivary composition is characteristic of the saliva of the caries-immune. This conclusion is in harmony with the findings of Miller that the incubation of a caries-susceptible saliva generates a higher percentage of acidity than the saliva of a caries-immune."

The separation of the mandibular saliva from the parotid saliva, by means of the saliva separator (6), enabled the author to obtain a satisfactory test for erythrodextrin or glycogen. If 2 cc. of mandibular saliva be collected and immediately slightly acidified and tested with 1 drop of freshly prepared N/10 iodine, a color characteristic of erythrodextrin or glycogen will be imparted to the mucin shreds suspended in the solution. If a larger quantity of mandibular saliva be collected and several drops of acetic acid added to each 5 cc. of saliva while collecting, to precipitate the mucin, and then filtered, the mucin precipitate will produce a dark reddish-brown color with iodine characteristic of erythrodextrin or glycogen. The difference in the color reactions of iodine with erythrodextrin and glycogen is not marked and it is sometimes difficult to distinguish between them.

If the saliva contains glycogen and enamel decalcification is the result of hydrolysis and fermentation of glycogen, the diastatic tests with starch, described in a previous report (7) may have no significance; the saliva would have to contain glycogenase. Accordingly, diastatic tests on the contrasting diets, similar to those made with starch, were made with glycogen, the results of which are shown in *fig. 1.* Exact comparisons with the diastatic tests with starch were impossible as it was necessary to change the concentration of the glycogen and the amount of saliva used in the tests, as the hydrolysis was too rapid when a glycogen solution of the same concentration as the starch and the same amount of saliva were used.

One gram of chemically pure glycogen was dissolved in 100 ml. of distilled water containing .5 gram of NaCl and boiled for 15 minutes, stirring constantly; 0.05 ml. of saliva was added to 5 ml. of the glycogen solution and placed in the incubator at 37°C. and spotted with 1 drop of freshly prepared iodine-potassium iodide solution. The end point was reached when all of the red or reddish-brown color disappeared and the solution remained a light straw color upon stirring.

Fig. 1 shows the results of these tests. The dotted lines indicate the diastatic action of the parotid saliva and the solid lines indicate

the diastatic action of the mandibular saliva. The hours of collection of the saliva are plotted on the abscissae and the time, in minutes,

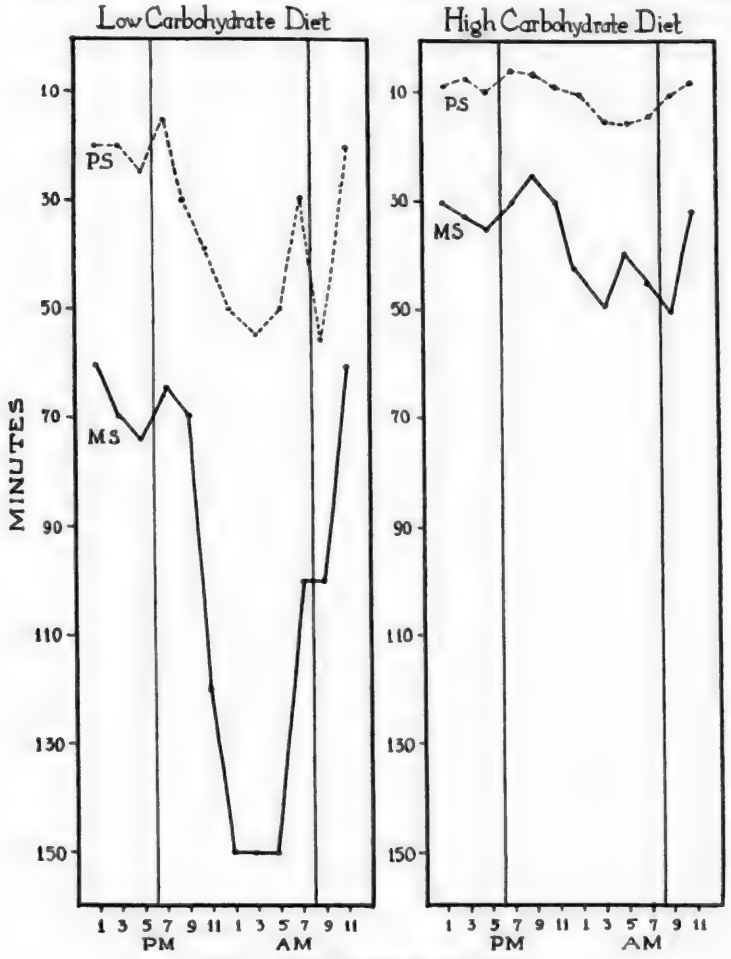

FIG. 1. Comparative diastatic tests

required to hydrolyze the glycogen to achroodextrin is plotted on the ordinates. The vertical lines indicate meal time. These charts not only show that a glycogenase is present in both the parotid and man-

dibular salivas and that hydrolysis of the glycogen does take place, but that it is much more active on a high carbohydrate diet than on a low carbohydrate diet. This is especially true of the mandibular saliva.

The minimum pH attained, in vitro, by the fermentation of carbohydrate in the author's saliva, incubated at 37°C., was pH 3.76. Controlled tests of the diastatic action of this saliva with glycogen show that the glycogenase is active at pH 3.70.

Numerous tests were made in an effort to isolate glycogen without success. A number of protein precipitants were tried in an effort to separate the carbohydrate and protein, but in each instance the carbohydrate was precipitated with the protein. Only by means of strong acids was it possible to disassociate the two groups, but this method hydrolyzed the carbohydrate into a reducing sugar and made the identification of the original carbohydrate impossible.

The degree of polymerization of the glucose molecule in the various polysaccharides is still a matter of controversy. Freudenberg (8) believes that starch contains 35 to 45 glucose units and glycogen contains approximately 12 to 18 units. Myrbäck (9) states "All stable dextrins have proved to be mixtures of a great number of saccharides of different molecular weight. In some cases, in spite of very thorough degradation, the staple dextrins contain from three (or possibly two) to about twenty glucose residues." According to Mathews (3) the starch molecule is 24 to 30 glucose molecules in length; the dextrins appear to contain 18 to 24 glucose units; achroodextrins contain 6 to 12 units and glycogen contains 12 to 18 glucose units.

Again quoting Mathews (3) "Many of the polysaccharides are quite stable in alkaline solution. This is the case for example, with glycogen or animal starch. It is prepared by destroying all the protein matter of the cells and all reducing sugars by cooking the tissue with 30 or 40 per cent potassium hydrate, a procedure which leaves the glycogen unaffected."

While the isolation of glycogen was not accomplished, certain corroborative evidence of the presence of glycogen was obtained. Using the saliva separator (6) 3 drops of a 30 per cent solution of KOH were placed in the mandibular test tube and 5 cc. of mandibular saliva collected, repeating until 60 cc. of mandibular saliva were collected,

the enzymes being immediately destroyed by the alkali. The 60 cc. of mandibular saliva plus approximately 2.3 cc. of KOH were evaporated over a water bath to approximately 2.3 cc. and kept at the boiling point for several hours, the volume being maintained. All reducing sugars and proteins were destroyed. After slightly acidifying a test with iodine gave the characteristic color reaction for erythrodextrin or glycogen. Attempts to precipitate the glycogen with 2 volumes of alcohol failed, but when the solution was neutralized with HCl and an excess of the acid added and boiled for several minutes, again neutralized and tested with Benedict's qualitative solution, a positive reaction for reducing sugar was obtained. Testing for reducing sugar before hydrolyzing with acid produced a negative result. Upon the addition of 2 volumes of alcohol a brown oily liquid settled to the bottom of the beaker, which upon hydrolyzing with acid, gave a positive reaction for reducing sugar.

The failure to isolate glycogen by the method described above may be accounted for as follows: In the hydrolytic cleavage of the glycogen molecule, as a result of the action of glycogenase, both secreted in the same glands, intermediate products between glycogen and erythrodextrin may be formed which have not been identified or isolated, and it is conceivable that the first stages of hydrolysis of the glycogen molecule may take place in the tubules and ducts of the salivary glands before the saliva enters the oral cavity.

If glycogen is present in the saliva, how did the glucose of the blood become converted into glycogen? In normal individuals when the blood sugar reaches a certain concentration the excess is converted into glycogen by the insulin secreted in the islands of Langerhans in the pancreas. Mathews (3) states "Insulin occurs not only in the pancreas but in other tissues of the body as well; and the salivary glands contain a considerable quantity of it"; "They (the salivary glands) contain considerable quantities of insulin"; "There is a marked increase in consumption of glucose on nerve and pilocarpine stimulation (Anrep) of the gland." Cahane and Cahane (15) stated that the removal of the sublingual gland of a dog produced a hyperglycemia.

In normal individuals the amount of insulin secreted is influenced by the amount of carbohydrate absorbed. Joslin (11) states "In passing it may be mentioned that Boller, Uiberrak and Falter have

demonstrated to their own satisfaction by means of blood transfusions, using insulin-sensitive recipients, that the blood of persons without metabolic disturbances contains minimal quantities of insulin when the person is fasting but larger amounts after a meal high in carbohydrate content."

If the amount of insulin in the salivary glands is proportional to the amount of insulin secreted, the carbohydrate in the saliva is probably proportional to the amount of insulin secreted in the islands of Langerhans, and susceptibility or immunity to caries depends on the activity of the island cells in the pancreas, which in turn, are influenced by the amount of carbohydrate absorbed. Should the island cells cease to function normally the diet ceases to be a determining factor in immunity or susceptibility and this may account for some of the cases that can not be traced to the diet. Joslin (17) states "Kent finds that although, as stated above, the diabetic has less caries than the non-diabetic, he is more disposed to tartar formation and periodontoclasia." The low incidence of caries in diabetes would seem to offer additional evidence that insulin is a factor in the etiology of dental caries.

On the other hand we would expect to find an increase in the incidence of caries in hyperinsulinism. There is nothing in the literature substantiating this theory; on the contrary, Whipple (18), Wilder (19) and Harris (20) report no apparent increase in caries in a considerable number of cases of hyperinsulinism. The hypoglycemia accompanying this condition may account for the failure to correlate caries with hyperinsulinism. Furthermore, Joslin (21) states "With the data now available it seems logical to assume that in the depancreatized dog and in the human individual with spontaneous diabetes there exists not only an overproduction of glucose in the liver but also a diminished efficiency in the combustion of glucose in the tissues. The end-result is that the normal utilization of glucose is interfered with and proper storage as glycogen in the liver and muscles does not take place." In other words, paradoxically, insulin produces a polymerization of the glucose molecule and also, in some unknown manner, facilitates its degradation in the tissues. Further elucidation on this subject will be necessary before any conclusion can be reached.

Joslin (22) states "Kent believes that destruction of the teeth and

supporting processes is usually very active just prior to the onset of diabetes." The author has had several cases in his practice, of a sudden and violent onset of caries followed by diabetes, but believes that the destruction of the supporting tissues accompany the subsequent hypoinsulinism.

While recognizing the conflicting evidence presented above, there is considerable evidence that insulin is a factor in the etiology of caries, viz.: 1. A polysaccharide exists in the saliva combined with a protein in the form of mucin. 2. There is a marked increase in the consumption of glucose from the blood in passing through the stimulated salivary glands. 3. There is a considerable quantity of insulin in the salivary glands. 4. That the polysaccharide polymerized from the glucose in the salivary glands is probably glycogen is indicated by the chemical tests described above on the saliva as collected from the oral cavity.

Rosebury (3) states "The first and distinctly more common variety attacks the teeth of children and young persons predominantly, occurs either in pits and fissures of the crown, or in the neighborhood of points of contact of approximal teeth. The second and less common variety attacks the teeth principally of adults, appears in the gingival area of the crown or in exposed roots, produces a large aperture on the surface and penetrates at a varying rate, often very slowly."

Joslin (11) calls attention to the influence of age on carbohydrate tolerance and cites the glucose tolerance studies of John on 192 children and 1500 adults. Normal curves were obtained in 82 per cent of the children, but in only 62 per cent of the adults. The decreasing sugar tolerance accompanying increased age would indicate a decreasing amount of insulin secreted and increasing immunity to caries.

Considering the first type of caries described by Rosebury, we find that it occurs during the growing age when the islands of Langerhans are most active and the drain on the calcium and phosphorus is greatest. It also occurs in protected areas where the vicious cycle of hydrolysis and fermentation of the carbohydrate may be continuous as described in a previous report (1). During pregnancy and lactation we have the drain on the Ca and P but there may be no change in the amount of insulin in the salivary glands. As a result we might expect an increase of susceptibility to caries, but to a lesser degree than that

which exists during the growing period. Considering the second type of caries described by Rosebury, we can readily understand its delayed appearance to adult age and its slow progress, as the factors producing it are intermittent and the periods of activity of short duration, probably only a few hours each night, as mastication and prophylaxis daily interrupt its progress (1).

It must be borne in mind that the potential concentration of the H ions is only one factor in this complicated process of enamel decalcification. An adequate concentration of potential Ca and PO_4 ions in saliva, regardless of the concentration of the potential H ions would produce immunity to dental caries. This is probably an important factor in our failure to consistently correlate immunity or susceptibility to caries with the amount of carbohydrate in the diet.

SUMMARY

Evidence is presented that the amount of insulin secreted in the body may be an important predisposing factor in determining immunity or susceptibility to dental caries.

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AMOUNT OF ORGANIC MATTER IN ENAMEL FROM SEVERAL TYPES OF HUMAN TEETH[1]

MARTIN DEAKINS, D.D.S., Ph.D.[2] AND J. F. VOLKER, D.D.S.

The Division of Dental Research, School of Medicine and Dentistry, The University of Rochester, Rochester, New York

Reports on the amount of organic matter in human enamel vary from 0 per cent to 7 per cent of the dry weight (1). There are two possible explanations for this disagreement; first, variation in the experimental procedures followed and second, actual variation in the amount of organic material in different types of teeth.[3] The latter possibility has been considered before (1, 2, 7) but the introduction of improved methods of sampling applicable to this problem makes the technics of previous investigators seem inadequate.

Experimental differences may be due to several factors, such as (a) *Method of drying the enamel.* Theoretically speaking, if protein is present the time and temperature must be the same for comparable results (3). The particle size should be approximately the same; powdered enamel may be more effectively dried than large pieces removed with a chisel. Vacuum drying is another condition which might yield different results expressed on a dry-weight basis. Karlström (2), however, could find no difference in weight loss of enamel samples dried *in vacuo* or otherwise. (b) *Manner of sampling.* Investigators have removed the outer layer of enamel with acid (4) or "brushed off Nasmyth's membrane" (1) before taking their samples for analysis. It is highly questionable whether these 2 treatments would give the same results. The acid-removable surface film cannot be removed by vigorous hand brushing (5). Removal of the surface

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[2] Now at School of Dentistry, University of Pennsylvania, Philadelphia.

[3] The commonly used method for determining "volatile materials" by incineration with addition of HNO_3 is not considered here; HNO_3 removes CO_2 which is present in enamel to the extent of 3–4 per cent (6,7) and incineration may or may not be complete without it.

film would probably change the *average* organic content (8), as determined by nitrogen analysis and expressed as proteins, since the acid-removable film has been found to contain 12 per cent nitrogen (9). In previous investigations chisels (1, 4) and burs (2, 10) have been used to obtain samples of certain portions of the enamel for analysis, with or without removing the surface layer. All these methods have the disadvantage that the sampling is neither representative nor reproducible.

The possibility that different types of teeth (deciduous, permanent, carious, unerupted, etc.) may have varying amounts of nitrogen-

TABLE I

Percentage of protein in enamel from various types of human teeth, calculated from the amount of total nitrogen

TYPE OF TOOTH	NO. OF TEETH IN GROUP	PERCENTAGE OF PROTEIN (TOTAL NITROGEN × 6.25)
1. Carious, deciduous	50	1.27
2. Carious, young, permanent	50	0.49
3. Carious, old, permanent	60	1.36
4. Non-carious, old, permanent	60	1.75
5. Non-carious, unerupted, permanent	15	1.95
6. Rat incisors*		
A. normal	30	2.97
B. fluorosed	30	2.59

* The rat teeth were from adult white rats weighing 250 gm. each. Fluorosis was produced by feeding 3 mg. of F per day per rat for 200 days (19).

containing material has not been investigated with adequate methods. Accordingly, the following experiment was undertaken to decide if such variation could be shown when a uniform and reproducible method was applied to pooled samples of these various types.

METHOD

Preparation of the enamel samples. The teeth were selected, cleaned by brushing, and grouped as shown in Table I, the anatomical distribution within the groups being practically the same (11). After the carious portion of the enamel from carious teeth was discarded, the teeth were allowed to dry and all the sound enamel was flaked off with a hammer and chisels, pooled and powdered in a steel mortar. The enamel powder was separated from any contaminating dentin by Manly's flotation method (12), using a liquid of density 2.65 for the first separation and of 2.80 for further purification of the enamel fraction. This manner of separation recovered 91 per cent of the enamel with a

purity of 99.4 per cent (12). The enamel thus obtained was washed free from the flotation liquid with acetone, dried and screened. All powder passing a 200-mesh screen was collected, further dried to constant weight at 110°C., and stored over $CaCl_2$.

Nitrogen determinations on the enamel. For the nitrogen determinations samples of about 100 mg. were weighed on the microbalance, and digested in 0.2 cc. of concentrated H_2SO_4 to which was added a drop of 0.1 per cent $CuSO_4$ and a small crystal of K_2SO_4. The digestion was carried on until the solution became clear and if it was colored, a micro-drop of 10 per cent H_2O_2 was added and the digestion continued until the solution became clear and colorless. The digest was made up to 10 cc. and triplicate aliquots taken for determination of nitrogen (13). The nitrogen values obtained were multiplied by 6.25 and are reported here as percentages of protein in the dried enamel (Table I).

For enamel studies, the sub-micro nitrogen method (13) is preferable to the ordinary micro-Kjeldahl method of Pregl (14) which has been used heretofore (1, 2, 7). The Pregl method is not reliable for quantities of nitrogen less than 0.2 mg. (15, 16) yet Karlström's samples contained even less than that. (They ranged from 0.0182 to 0.1876 mg. of nitrogen.) Similarly, Murray and Bowes report a difference of 0.009 per cent in the nitrogen contents of normal and "pyorrhetic" enamel (7), that is, a difference of 1 part in 10,000. This difference is not significant, considering the amount of material used and the limitations of micro methods for nitrogen determination. These criticisms show the general temptation to express nitrogen values in too many decimal places.

DISCUSSION

The variation of nitrogen-containing organic material on enamel from different types of human teeth, is apparent in Table I. Considering the fact that one procedure was used throughout, and that enough teeth were used to average out the small anatomical differences in nitrogen contents (2), these results show a real difference in the organic content of enamels. It should be emphasized that nearly all the enamel (90 per cent) from each tooth was used (excepting the carious) and that at least 15 teeth were pooled for sampling in each group. This procedure, we believe, insured random sampling of the powdered material for the nitrogen analyses.

It will be seen in Table I that the range of enamel protein in human teeth is from 0.49 to 1.95 per cent, or 1.46 per cent. This great variability is much more than that found in dentin (17). It revives the question of whether the nitrogen-containing fraction of the organic matrix is a structural element of the tissue, or an epithelial residue, as has been postulated before (4).

In general, results are higher than others based on nitrogen determinations (1, 2, 4). This difference may be due to (a) our use of a high percentage of the enamel without removing the outer layer, and (b) more effective drying of the enamel as a result of its being finely

powdered. However, our values agree substantially with other recent studies made on powdered enamel, separated from dentin by flotation (18), both in the range of values and their magnitude. Fluorosed rat enamel from incisors contained relatively 10 per cent less organic matter than the normal root enamel, showing that fluorine causes hypercalcification of rat enamel as well as bone.

It is interesting, if not significant, that the enamel of non-carious teeth contains higher organic percentages than the non-carious enamel from carious teeth. This may or may not be a true difference. Karlström (2) found the opposite. There seems to be no relationship between the organic content as measured here and the solubility of the enamel in the various groups (11). Unfortunately, "non-carious, young, permanent" and "non-carious, deciduous" groups were not available for analysis.

SUMMARY

The average organic content of various types of human enamel, based on total nitrogen determinations, was found to be 0.49 per cent to 1.95 per cent. Rat incisor enamel from normal and fluorosed animals, analyzed by the same method, had 2.97 per cent and 2.59 per cent respectively.

The method employed made use of reproducible technics throughout, and insured random sampling of the material.

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COMPARATIVE MEASUREMENTS OF SALIVARY pH[1]

GEORGE D. WESSINGER, PH.D.

From the Foundation for Dental Research of the Chicago College of Dental Surgery, Chicago, Illinois

Some previous investigations by the author (1) have demonstrated that there are reducing compounds in saliva which can serve as hydrogen donors for the reduction of pyruvic to lactic acid under the influence of enzymes contained in yeast and other mouth organisms. A further investigation into this interesting observation seemed desirable because of the influence that these substances might have on the determination of various properties of this important fluid.

The quinhydrone electrode has been applied to the determination of the hydrogen ion concentration of a variety of solutions. It was first believed that this electrode functioned as a hydrogen electrode at exceedingly low pressures of hydrogen, but it has been definitely established now that one is dealing with an oxidation-reduction reaction. In solution quinhydrone dissociates into quinone and the readily oxidized hydroquinone; but the ratio between these two products is controlled by the pH of the solution and the electrical potential of the immersed inert electrode is fixed by this ratio. Now if any factor other than pH affects this ratio an error will result and there are several such factors that may enter. Thus if the solution contains oxidizing or reducing substances (2, 3) of proper potential this ratio may be changed and thus preclude the possibility of an accurate estimation of pH.

Because of the fact that pH measurements on saliva by the quinhydrone method have appeared in the literature from time to time, it seemed desirable to present some data on the relative differences, if any, obtained by this method as compared with the glass electrode. The glass electrode is considered by most investigators to be consistently more accurate in solutions of unknown composition because

[1] (Received for publication January 3, 1941.)

it is said to be unaffected by the various factors which cause errors in the other methods. It has been stated in the literature (4) that the quinhydrone method is unreliable for the determination of salivary pH. This was found to be true if a voltmeter of the type designed for this purpose is used or if the simple potentiometric method is employed. Considerable drift in potential was noted making it very difficult to obtain a stable point of equilibrium. The possibility at once suggested itself that this drifting of potential might possibly be due to polarization of the electrode. To test this thesis, a vacuum tube potentiometer of the type ordinarily used with glass electrodes was next employed. Since the function of a vacuum tube potentiometer is to cut down to a minimum the flow of current to the electrode, polarization is also reduced to a minimum. In the investigation reported on herein, the pH of a number of samples of saliva has been determined by both the glass and quinhydrone electrodes and arranged in a table for purposes of comparison.

In the quinhydrone method an inert electrode of gold electrodeposited on platinum was used. This seemed desirable because of the low catalytic effect of gold as compared to platinum. A saturated calomel half-cell and a saturated potassium chloride bridge was employed to complete the cell and the potential measured in all cases by means of a vacuum tube potentiometer. Measurements by the glass electrode were made with a Coleman pH electrometer, model 3D. Standard acetate and phthalate buffers were used to check both methods against each other in order to insure proper working conditions and to preclude the possibility of any differences due to inherent error in the apparatus. In all cases the 2 methods were checked against each other just before measurements on saliva were made.

For the present work, unstimulated saliva was used. The saliva was obtained from patients selected at random and referred to the author by the Diagnostic Department of the clinic of the Chicago College of Dental Surgery. Most of the samples represent what is commonly called "resting saliva." The sample was divided into 2 portions for pH measurements by the 2 methods. Quinhydrone in excess of that necessary to saturate the saliva was added to one sample and the pH of the other sample measured with the glass electrode.

The pH of the other sample was then measured immediately by the quinhydrone method. Table I shows the results on the saliva of fifty patients. It will be observed that the differences between the 2 methods vary somewhat. In some instances the correspondence is quite close and in no case was there obtained differences of more than

TABLE I

Comparison of pH determinations on 50 patients

pH, QUINHYDRONE ELECTRODE	pH, GLASS ELECTRODE	DIFF.	pH, QUINHYDRONE ELECTRODE	pH, GLASS ELECTRODE	DIFF.
6.70	6.77	−0.07	6.71	6.76	−0.05
6.81	6.90	−0.09	6.77	6.84	−0.07
6.93	7.05	−0.12	6.90	6.92	−0.02
6.84	6.95	−0.11	7.15	7.12	+0.03
6.93	7.03	−0.10	6.64	6.73	−0.09
7.20	7.12	+0.08	6.75	6.81	−0.06
7.27	7.20	+0.07	6.90	6.94	−0.04
6.88	6.97	−0.09	6.81	6.88	−0.07
6.93	6.99	−0.06	6.65	6.77	−0.12
6.70	6.79	−0.09	7.18	7.25	−0.07
6.79	6.85	−0.06	6.15	6.16	−0.01
6.87	6.95	−0.08	6.78	6.88	−0.10
6.52	6.61	−0.09	6.51	6.63	−0.12
6.74	6.70	+0.04	6.65	6.71	−0.06
7.01	7.09	−0.08	6.83	6.95	−0.12
6.77	6.84	−0.07	6.25	6.31	−0.06
6.64	6.73	−0.09	6.44	6.47	−0.03
6.82	6.93	−0.11	6.63	6.60	+0.03
6.71	6.84	−0.13	6.73	6.85	−0.12
6.48	6.57	−0.09	6.50	6.60	−0.10
6.77	6.87	−0.10	6.42	6.52	−0.10
6.75	6.81	−0.06	6.74	6.83	−0.09
6.41	6.49	−0.08	6.15	6.23	−0.08
6.29	6.38	−0.09	7.22	7.10	+0.12
6.97	7.09	−0.13	6.64	6.71	−0.07

0.13 pH units. It will also be noted that the values obtained by the quinhydrone method are rather consistently lower than with the glass electrode.

Table II shows measurements on 3 individuals in which several measurements were made on different days. It will be noted here that on the limited number of cases tried that the differences obtained

on any one individual were not consistent but vary somewhat from day to day.

Although the differences obtained by the 2 methods were not large, it seemed of interest to measure the oxidation-reduction potentials of some of the samples and determine if any correlation between this value and the difference in pH by the 2 methods could be established. To the author's knowledge, no measurements of the oxidation-reduction potentials of saliva have as yet appeared in the literature. For these measurements a gold electrode was immersed in saliva and the half-cell thus formed was balanced against a saturated calomel half-

TABLE II

Comparison of reported pH determinations on 3 individuals

	DATE	pH, QUINHYDRONE ELECTRODE	pH, GLASS ELECTRODE	DIFF.
Case I	1/2/40	6.70	6.77	−0.07
	1/3/40	7.05	7.10	−0.05
	1/8/40	6.80	6.77	+0.03
	3/6/40	6.93	7.03	−0.10
Case II	1/2/40	6.81	6.90	−0.09
	2/6/40	7.11	7.12	−0.01
	2/8/40	6.78	6.81	−0.03
Case III	1/3/40	6.60	6.62	−0.02
	1/8/40	6.82	6.88	−0.06
	2/8/40	6.91	6.87	+0.04

cell by means of a vacuum tube potentiometer. The 2 half-cells were connected by means of a saturated potassium chloride bridge. The E.M.F. seemed to change very rapidly and the redox potential appeared to become more negative as time elapsed. In many cases it was almost impossible to obtain a stable point of equilibrium; even when an equilibrium point had been approximated it changed appreciably within 1 minute, always in a negative direction. Apparently the oxidation-reduction system in saliva is very poorly poised. Collecting the samples and measuring the potentials under mineral oil did not eliminate the drift. Because of this constant and appreciable drift in potential, no accurate data on the oxidation-reduction poten-

tial of saliva can be presented at this time. It seems probable that accurate measurements of the redox potential of saliva will be very difficult to make, due, apparently, to the poorly poised oxidation-reduction system in saliva. This is analagous to an attempt to measure the pH of unbuffered solutions. Polarization of the electrode might also be considered as a contributing factor to the rapid drift of potential but this seems very remote; the vacuum tube potentiometer employed was an impulse amplifier and was so constructed as to allow only minute quantities of energy to flow through the electrode and only then for a small fraction of a second.

SUMMARY

1. The drift of potential frequently encountered in measuring the pH of saliva by the quinhydrone method can be eliminated by the use of a vacuum tube potentiometer.

2. The salivary pH of 50 patients was measured by means of the glass and quinhydrone electrodes for purposes of comparison. The quinhydrone method usually gave slightly lower results, the greatest deviation being 0.13 pH units and the average for 50 measurements was about 0.08.

3. The differences in salivary pH as measured by the 2 methods was not constant for any one individual but varied from day to day.

4. Attempts to correlate the difference in pH by the 2 methods with oxidation-reduction potentials were unsuccessful because of the difficulties encountered in measuring the latter. From theoretical considerations, however, we can say that the deviation in pH by the quinhydrone method will probably be greater the farther removed the oxidation-reduction potential is from zero.

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EFFECT OF STORAGE TEMPERATURE ON THE PRECIPITABLE PROTEIN CONTENT AND CATALASE ACTIVITY OF WHOLE SALIVA[1]

MARTIN DEAKINS, D.D.S., PH.D.[2]

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, New York

Becks (1) has called attention to the need for uniform preliminary treatment of saliva after collection and before making any chemical analyses. For example, he has shown that centrifugation, deproteinization, or both, produce errors in calcium determinations on whole saliva. When using the Halverson-Bergeim method for calcium (2), combinations of the two treatments just mentioned gave increasing values in the following order: 1. Preliminary centrifugation, no deproteinization. 2. Preliminary centrifugation, deproteinization. 3. No preliminary centrifugation, deproteinization. 4. No preliminary centrifugation, no deproteinization. In fact, centrifugation alone caused an average decrease of 6 per cent in the calcium values of the same sample of saliva analyzed by the same method and investigator. This effect seemed to be due to removal of the proteins. If the amount of protein in saliva influences the calcium determinations, it may also affect the other inorganic analyses. Hence, any other factors affecting the protein content (e.g., proteolysis) become important considerations in salivary analyses.

Salivary proteins may be broken down either by a proteolytic enzyme which exists in saliva (3, 4) or by bacterial decomposition. Toluene and chloroform prevent bacterial decomposition and are commonly used for that purpose, but these compounds do not prevent breakdown of the proteins by the auto-proteolytic action of the saliva (5, 6). Consequently, the precipitable protein content will decrease when saliva is kept for some time at room temperature, either with or

[1] This investigation was supported by a grant from the Carnegie Corporation of New York. (Received for publication October 24, 1940.)

[2] Now at School of Dentistry, University of Pennsylvania, Phila., Pa.

without these preserving agents (6). Similarly, there is a change in the amount of catalase activity in saliva when kept for some time at room temperature.

The purpose of this investigation is to determine if these changes in protein content and catalase activity are great enough to necessitate special care in the preliminary treatment of saliva collected for chemical analysis.

METHODS AND RESULTS

Whole saliva, unstimulated, was collected in a 50 cc. centrifuge tube surrounded by chipped ice and water. The rate of flow was 37.3 cc. per hour. After mixing well, duplicate 2 cc. aliquots were taken immediately after collection and at later intervals for protein nitrogen determinations[3] by the micro-Kjeldahl method (7). Simultaneously, duplicate 1 cc. aliquots were taken for catalase determinations.[4] The remaining saliva was left in the ice-water bath and set in the ice box, thus maintaining the temperature at 0° C. Both the protein and catalase determinations were repeated at the intervals shown in *figs. 1* and *2*. Neither the precipitable protein nor catalase activity had changed significantly after 28 hours at 0° C.

A second sample of saliva was collected some days later in the same manner from the same subject (M. D.) under the same conditions, at the rate of 47.2 cc. per hour. It was kept on ice as before until the whole sample was collected, mixed well, and duplicate aliquots taken immediately for protein and catalase determinations. The remainder was heated to 30° C. (room temperature) and kept at this temperature for the rest of the experiment. Analyses were repeated at the times shown in *figs. 1* and *2*. It will be seen that the total protein nitrogen of whole saliva decreased from 43 to 25 milligrams per cent in 24 hours, a relative *decrease* of 42 per cent. The catalase activity in the same sample changed from 9.9 to 4.8 (values are cc. of 0.1 N $KMnO_4$), a relative *increase* of 51.5 per cent. This is an increase in activity, for these figures are volumes of $KMnO_4$ necessary to titrate the undecomposed H_2O_2 remaining in the saliva-H_2O_2 mixture after incubation at room temperature. The increase in catalase is probably due to the presence of certain aerobic bacteria (8).

A third sample (rate of flow, 46 cc. per hour) was collected over ice, centrifuged immediately for 10 minutes at 2000 R.P.M., and duplicate aliquots taken from the supernatant liquid for protein determinations. It was then heated to 30° C. and kept at that temperature for the remaining analyses. No catalase determinations were made on this

[3] Proteins were precipitated by adding to the saliva an equal volume of 1.6 per cent uranyl acetate. The precipitate was then washed 3 times with an 0.8 per cent solution of uranyl acetate, digested, and steam distilled.

[4] To determine the catalase activity, 3 cc. of 0.66 molar H_2O_2 were added to 1 cc. of saliva and the mixture incubated for 1 hour at 37° C. 1 cc. of 20 per cent H_2SO_4 was then added and the undecomposed H_2O_2 titrated with 0.1 N $KMnO_4$. A blank determination was made simultaneously under the same conditions on another cc. of the saliva. Here, however, the H_2SO_4 was added to the mixture before incubation to kill all enzyme activity. The difference between the amounts of $KMnO_4$ needed to oxidize the blank and the sample represents the amount of H_2O_2 decomposed by the catalase.

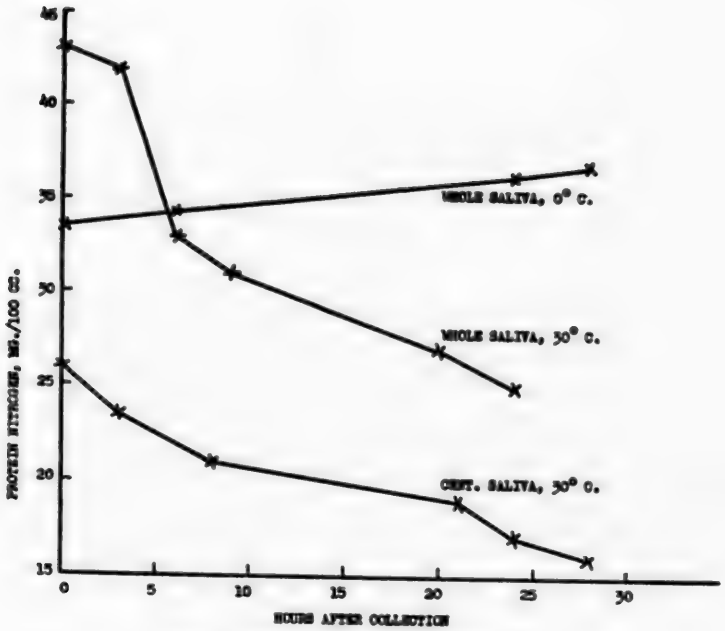

FIG. 1. Precipitable protein in whole saliva

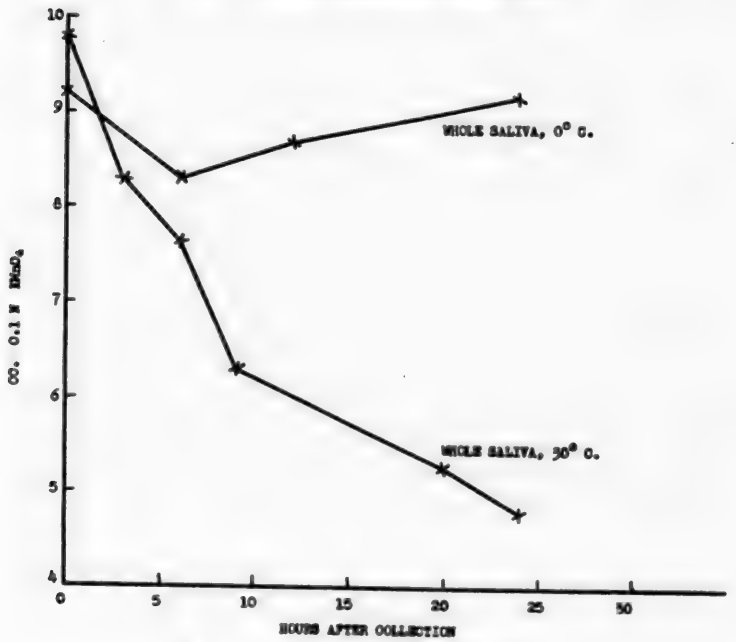

FIG. 2. Catalase activity in whole saliva

sample. The decrease in protein content was from 25.8 to 17.0 milligrams per cent in 24 hours, a relative decrease of 34.1 per cent (*fig. 1*). There is, therefore, a smaller relative decrease in the protein content of centrifuged saliva than of whole saliva when both are kept at 30° C.

In all cases the rate of protein breakdown is greatest during the first 8 hours after collection.

CONCLUSIONS

These results indicate that the amounts of protein nitrogen, non-protein nitrogen, and catalase activity of saliva are indeed appreciably affected by storage at room temperature. If this change in the protein content affects the calcium determinations (1), one may suppose that other analyses are likewise influenced. The fact that catalase activity is also greatly changed is further evidence that all samples of saliva, whether collected for analysis of chemical constituents or enzyme studies, should be both collected and stored at 0°C.

SUMMARY

The effect of storage of saliva at room- and at icebox-temperatures has been studied. The protein content of whole saliva decreased 42 per cent on standing 24 hours at 30°C.; its catalase activity was doubled. The protein content of centrifuged saliva decreased 34 per cent under the same conditions. At 0°C. no significant change took place either in the amount of protein or catalase activity.

Evidence is presented that saliva should always be collected in containers at 0°C., even for immediate analyses.

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THE INCIDENCE OF ROOT RESORPTION OF VITAL PERMANENT TEETH[1]

SAMUEL HEMLEY, D.D.S.

Department of Orthodontics, New York University, College of Dentistry

Orthodontic therapy was instituted without any great fear of dire consequences until the report by Ketcham in 1929 which showed a rather high incidence of root resorption on vital permanent teeth. As a result of Ketcham's investigations, it was revealed that root resorption occurred in about 1 per cent of cases that were never subjected to orthodontic treatment and that the number of cases rose to 21 per cent during or following orthodontic therapy. More recently, Rudolph (1936) reported that the incidence of root resorption was as high as 74 per cent during or following orthodontic treatment, and in 1940 he reported that even this high incidence was often exceeded. The attempts to explain the etiology of this manifestation have been varied: Marshall claimed that it is due to dietetic disturbances. Becks believed that it is due to endocrine disturbances. Oppenheim accused the shapes of the roots of certain teeth. With this apparent disagreement there has been a prevailing concept both among the professions and the laity that root resorption is something over which the orthodontist has no control and it is one of the inevitable dangers incidental to orthodontic therapy.

It was the desire to allay fear in this connection and also the desire to study the etiology of root resorption that led to this investigation. A study of the manner of growth of the jaws and tissue changes incidental to tooth movement led to the conclusion, as reported by the writer in 1937, that horizontal movements of the apex in bone predispose the tooth to root resorption. As was explained at that time, in accordance with the findings of Oppenheim, when a gentle intermittent force is used, the tooth becomes a one-armed lever with the

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apex as a fulcrum. If, however, excessive force or continuous force is used, the root becomes a two-armed lever with the apex moving in a direction opposite to that of the crown. Even when gentle intermittent force is used, certain appliances are designed to move the apex horizontally through bone. Accordingly, therapy on 195 consecutive cases was instituted, either with the attempt to avoid movement of apices horizontally through bone, or, in those cases in which it was felt that a horizontal movement was necessary, every effort was made to accomplish this movement as slowly as possible.[2]

Of the 195 cases that were treated, 121 (62.1 per cent) were completed, the other cases dropping out after an interval of treatment for reasons beyond our control. These patients were treated at the New York University, College of Dentistry in the Department of Orthodontics by the post-graduate students. It might be mentioned here that after a period of three months of didactic training, patients are assigned to the post-graduate students. Only new cases are assigned to them and they continue treatment, if need be, over a period of as much as 4 years so that, with rare exceptions only, the one man treats the patient throughout the entire course of treatment.

Up to the present time a case was listed as having root resorption if the radiographs showed manifestations of this phenomenon involving any of the teeth. As a result, a patient with but a minute blunting of the apex of one tooth and a patient having extensive root resorption of all of the teeth could not be differentiated in the reports thus far submitted. If we are to study this phenomenon with the hope of determining its cause, it is obviously of great interest to know not only the teeth involved but also the extent of the root resorption.

In spite of the difficulties involved in differentiating the degree of

[2] In this connection, it should be noted that Angle in 1900, made the statement that, "The malpositions of the teeth consist principally in the variation from the normal of the positions of their crowns, with usually little displacement of the apices of their roots, so that they incline at an angle more or less oblique from the normal. In some instances, however, there is some displacement of the apices as well as of the crowns, they having either developed in malpositions, or having, as in most instances, been forced from their normal positions by the eruption of more powerful teeth in juxtaposition."

While it is a fact that he later introduced appliances to produce root movement, no evidence was ever presented by anyone to disprove the opinion expressed above. Indeed, the madder feeding experiments by Brash conclusively demonstrated that roots do not migrate horizontally through bone and furnished proof to justify Angle's hypothesis.

root resorption, it was felt that some arbitrary divisions were important so that we might analyze, not only the incidence of root resorption but the degree of root resorption. With this in mind, there were 4 different degrees of root resorption noted. In the first stage, designated slight, there was merely a blunting of the apices of the teeth (*fig. 1*). In the second stage, designated as moderate, there was

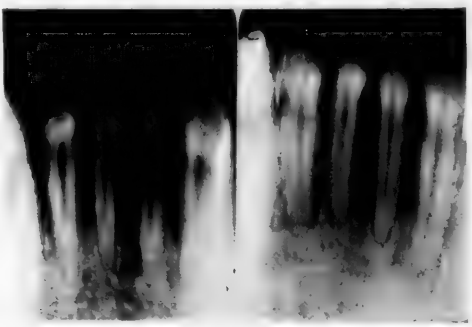

FIG. 1. Radiographs, before and after orthodontic treatment. There is blunting of apices and this degree of resorption is designated "slight."

FIG. 2. Radiographs, before and after treatment. This stage, designated as "moderate" includes all teeth which show loss of root from slight resorption to just less than one third of root.

a loss of the apices to the extent of from slight to that involving less than $\frac{1}{3}$ of the length of the root, (*fig. 2*) and in the third stage, here designated as medium, the extent of root resorption was approximately $\frac{1}{3}$ of the root, (*fig. 3*), and all cases in which the resorption would exceed $\frac{1}{3}$ of the root, (*fig. 4*), were to be listed in the category of marked root resorption. Fully conscious of the difficulties involved in such an arbitrary classification, where no measurements are readily

available, the necessity for this classification was so obvious that in classifying cases, every effort was made to lean backward in the de-

FIG. 3. Radiographs before and after orthodontic treatment. When the extent of root resorption is approximately one third the case is designated as "medium."

sire to make our findings absolutely authentic. Radiographs were taken before treatment and at least once a year thereafter. Three

men independently examined the radiographs of every case, one of these men being an anthropologist and the two others orthodontists.

In any case in which there was a disagreement, the case was automatically put into the classification of the more severe type of root resorption. Likewise, in many cases where the orthodontist felt there was merely a widening of the periodontal space with no root resorption in which however, the anthropologist felt there may be some root resorption, the cases were classified as slight root resorption.

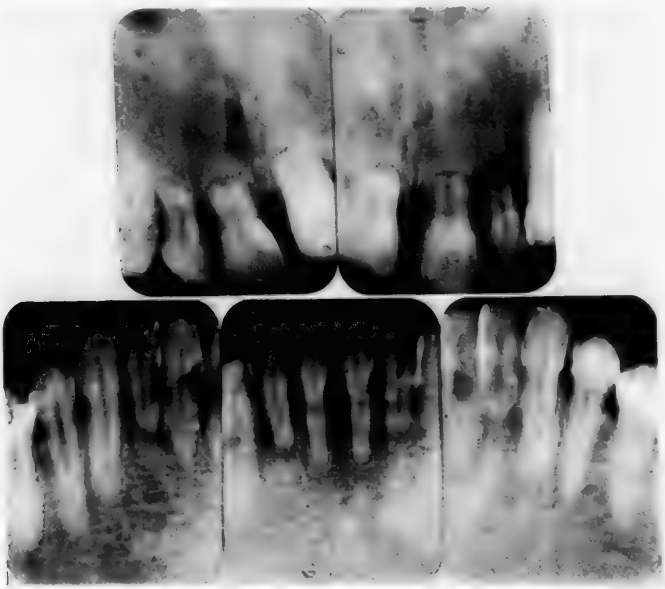

FIG. 4. "Marked" root resorption. More than one third of the root has been resorbed. This patient was treated with a pin and tube appliance from 1923 to 1932.

Only in those cases where there was no question at all in the minds of all 3 men were the cases listed as having no root resorption. Forty-two (21.5 per cent) of the 195 cases that were treated showed root resorption. It is of interest to note that this is almost exactly the percentage reported by Ketcham (21 per cent). In the 42 cases which showed root resorption, an analysis of the teeth reveals that the incidence of root resorption was as follows: 68.6 per cent, slight; 26.7 per cent, moderate; 4.7 per cent, medium; 0.0 per cent, no cases

marked. There are all told only 172 teeth that showed root resorption and the total number of teeth subjected to treatment was 4,959; that is, only 3.5 per cent showed any evidence of resorption.

Of the 42 cases which showed root resorption, 16 (38 per cent) were males and 26 (62 per cent) were females. Of the entire group of 195 cases, 36 per cent were males and 64 per cent were females. The average time of treatment in the root resorption cases was 2 years 8 months with a standard deviation of 10.3 months. The minimum period of treatment was 11 months and the maximum was 51 months. The average age of the root resorption cases was 15.2 years with the standard deviation of 3.95 years.

TABLE 1

Per cent of root resorption in all teeth present in cases treated orthodontically (195)

	FIRST INCISORS	SECOND INCISORS	CANINES	1 Pm s	2 Pm s	1 M s	2 M s	TOTAL
No. of teeth	377	381	389	387	384	376	194	2488
Upper	7.4	10.0	1.5	2.8	1.6	1.1	0.5	3.7
No. of teeth	389	388	389	387	381	343	194	2471
Lower	11.1	8.0	0.3	0.0	0.0	1.2	0.0	3.2
Both	9.1	9.0	0.9	1.4	0.8	1.1	0.3	3.5

It was further desired to determine the percentage of the particular teeth which showed root resorption. In order to obtain the number of teeth which were subjected to root resorption, all missing teeth were deducted from the total number of teeth. A tooth was counted as missing whether it had been extracted, congenitally missing, impacted or not yet erupted at the time the case was started. This last stage involved essentially third permanent molars and in some cases second permanent molars because of our practice of delaying treatment, whenever possible, until the loss of all of the deciduous teeth. The addition of all permanent molars would reduce the percentage of incidence of root resorption. It will be noted from Table I that the teeth most susceptible to root resorption are, in order of listing: 1. The incisors, 9.1 per cent first incisors, 9.0 per cent second incisors. 2. First premolars, 1.4 per cent. 3. First permanent molars, 1.1 per cent. 4. Canines, 0.9 per cent. 5. Second premolars, 0.8 per cent.

6. Second molars, 0.3 per cent. However, there is considerable difference in the susceptibility of the maxillary and of the mandibular teeth. For example, the maxillary first incisor is much less susceptible than the mandibular first incisor; while the maxillary second incisors are much more susceptible than the mandibular second incisors. The maxillary canines show a much greater susceptibility than do the mandibular canines. There were no resorptions on the mandibular premolars and in the upper jaw it was found that the first premolar is much more susceptible than the second premolar.

The first permanent molars are of particular interest because they have been used, traditionally, for anchorage and we find that there is relatively little difference between the maxillary and mandibular first permanent molars, the maxillary being slightly less susceptible. There is a small amount of resorption of the maxillary second molars and no resorption of the mandibular second molars.

It is further of interest to determine the amount of resorption which develops on each of the teeth involved. Table II gives this data. Of the 42 cases which showed evidence of root resorption, 78.6 per cent were completed cases. It will be noted that only 62.1 per cent of the 195 cases were completed, but that the percentage of completed cases is much higher in the group which had root resorption. An analysis of the 172 teeth which showed resorption reveals that the right and left sides are almost identical. In the upper jaw slightly more than half of the teeth involved, 51.2 per cent on the upper left and 52.0 per cent on the upper right, were only slightly involved, that is, there was a mere blunting of the apices, and in the lower jaw 88.6 per cent of the teeth involved were only slightly involved. In the upper jaw those teeth which were moderately involved, that is, where less than $\frac{1}{3}$ of the root was resorbed included 39.5 per cent on the left and 40.0 per cent on the right. In the lower jaw, the teeth which were moderately involved included 11.4 per cent of the teeth involved. Only those teeth which are listed as medium, those teeth in which there was resorption of the roots up to approximately $\frac{1}{3}$ of the root, are of any real significance because of the fact that only in these cases do we begin to lose a sufficient portion of the root to cause us any concern. When more than $\frac{1}{3}$ of the root is lost there may perhaps be danger to the investing structures because of the relatively longer

lever arm of the crown as compared to that of the root of the tooth. It will be noted that teeth involved sufficiently to be classified as medium root resorption, those cases approaching a degree of resorption which may endanger the serviceability of the teeth, are, on the maxillary left side: One first incisor, one second incisor, one canine, one

TABLE 2

Distribution of the 172 teeth which showed radiographic evidence of root resorption

TOOTH AFFECTED	LEFT						RIGHT						L + R	
	sl.	mod.	med.	mkd.	Total No.	Total Per cent	sl.	mod.	med.	mkd.	Total No.	Total Per cent	No.	Per cent
Upper arch														
First incisor	5	7	1	0	(13)	30.2	7	6	1	0	(14)	28.0	(27)	29.0
Second incisor	11	4	1	0	(16)	37.2	14	7	1	0	(22)	44.0	(38)	40.9
Canine	1	1	1	0	(3)	7.0	0	3	0	0	(3)	6.0	(6)	6.5
Premolar I	1	1	1	0	(3)	7.0	2	4	2	0	(8)	16.0	(11)	11.8
Premolar II	1	3	0	0	(4)	9.3	2	0	0	0	(2)	4.0	(6)	6.5
Molar I	2	1	0	0	(3)	7.0	1	0	0	0	(1)	2.0	(4)	4.3
Molar II	1	0	0	0	(1)	2.3	0	0	0	0	—	—	(1)	1.0
Total	22	17	4	0	(43)	100.0	26	20	4	0	(50)	100.0	(93)	100.0
Per cent	51.2	39.5	9.3	—		100.0	52.0	40.0	8.0	—		100.0		
Lower arch														
First incisor	18	3	0	0	(21)	60.0	19	3	0	0	(22)	50.0	(43)	54.4
Second incisor	10	1	0	0	(11)	31.4	18	2	0	0	(20)	45.5	(31)	39.2
Canine	1	0	0	0	(1)	2.9	0	0	0	0	—	—	(1)	1.3
Premolar I	0	0	0	0	—	—	0	0	0	0	—	—	—	—
Premolar II	0	0	0	0	—	—	0	0	0	0	—	—	—	—
Molar I	2	0	0	0	(2)	5.7	2	0	0	0	(2)	4.5	(4)	5.1
Total	31	4	—	—	(35)	100.0	39	5	—	—	(44)	100.0	(79)	100.0
Per cent	88.6	11.4	—	—		100.0	88.6	11.4	—	—		100.0		

first premolar. On the upper right side they are: One first incisor, one second incisor, two first premolars. In the lower arch no teeth were involved to this degree.

It should be remembered that all the teeth which showed a mere blunting were listed as slight resorption. In all of these cases, if there is any actual loss of the apex of the tooth, it is so slight as to be

hardly appreciable. However, the teeth which showed sufficient root resorption to be classified as moderate and medium actually show a definite loss of the root. All of the teeth listed as slight root resorption are, as a matter of fact, so slight as to be practically negligible for this study. If, therefore, only those teeth which are listed as moderate and medium are counted the result shows: Maxilla: First incisors, 15; Second incisors, 13; Canines, 5; 1st Premolars, 8; 2nd Premolars, 3; 1st Permanent molar, 1. Mandible: First incisors, 6; Second incisors, 3.

SUMMARY

Of a total of 4,959 teeth exposed to orthodontic therapy, only 172 teeth (3.5 per cent) showed any evidence of root resorption. Of this group, only 8 teeth (0.2 per cent) involved as much as $\frac{1}{3}$ of the root, and no teeth suffered a loss of more than approximately $\frac{1}{3}$ of the root. It is thus apparent that the incidence of root resorption can be reduced to the extent that it need not be regarded as a hazard to orthodontic treatment.

Grateful acknowledgment is given to Drs. Adolph Jutkowitz, Irving M. Halperin and Marcus Goldstein of New York University, College of Dentistry for the work they have done in examining the records and classifying their findings.

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SULFONAMIDE CHEMOTHERAPY OF DENTAL INFECTIONS[1]

LEO STERN, D.D.S.

Dental Department, The Mount Sinai Hospital, New York City, N. Y.

INTRODUCTION

The extensive vogue attained by sulfanilamide and its derivatives in the practice of medicine naturally raises the question of the availability of these drugs in the management of dental diseases. Surprisingly little on this topic appears in dental literature (1), despite the fact that these drugs are considered particularly effective in controlling infections caused by some of the bacteria commonly identified with dental sepsis. Furthermore, the few published data concerning specific chemotherapeutics of oral infections are so loosely integrated that it seems appropriate at this time to indicate some criteria that should govern their acceptability in the practice of dentistry, and to suggest a simple but comprehensive pattern on which clinical reports may be based so as to permit fair comparisons.

A series of documented reports is projected intending to assay the value of the commonly used sulfonamide compounds, sulfanilamide, sulfapyridine and sulfathiazole, in the prevention and treatment of various types of infections arising from the teeth, jaws, and oral mucosa. This particular report deals with the chemotherapy of extraction wounds and other open contaminated wounds of the maxillary bones.

With the exception of salvarsan, the modern history of chemical specifics is one of repeated disappointments resulting from an uncritical acceptance of new remedies (2). In dramatic contrast, the concensus concerning sulfanilamide remains enthusiastic after more than five years' trial. Yet it is significant to note that interwoven among a preponderance of extravagant reports, many cautious and even dissenting opinions appear. The most recent literature embodies

[1](Received for publication December 10, 1941.)

more rigid, critical standards and pays considerable attention to the incidence and peculiarity of toxic reactions. The Council of Pharmacy (3) places exceptional emphasis on the need for caution in its administration.

Thus, it is evident that opinion regarding chemotherapy, while for the most part favorable, is still in a state of flux, a circumstance that should influence dental clinicians to qualify its adoption with reservations. A careful study of the sulfonamide compounds must be made by the dentist who prescribes them because he assumes the responsibility of recognizing and dealing with unpredictable toxic effects.

DENTAL PRESCRIPTION OF SULFANILAMIDE

Many physicians express the opinion that the function of prescribing general remedies, even those designed to correct dental disease, is outside the province of the dental clinician. Nevertheless, the dentist is licensed by law as well as privileged by custom to prescribe for the relief or cure of any lesion arising from the teeth or jaws (4). Unquestionably this prerogative extends to the use of the sulfonamides as well as to any other drug that may be useful, in terms of dental disease, in promoting the welfare of the patient. However, the clinician, either *dental or medical*, should limit himself to dispensing those drugs, of which the indications, limitations and characteristics are familiar to him. It need scarcely be emphasized that such restraint is particularly necessary in connection with the sulfonamides.

Because reactions to the sulfonamide compounds are often severe, because damage now unrecognized may be disclosed at some future time and because a competent daily check-up is the only reasonable safeguard to its use, this form of therapy in dentistry must still be regarded as experimental. Enteral administration of large doses of the sulfonamides is therefore warranted only in cases that are both carefully selected and which lend themselves to adequate supervision. The ideal set-up for these restrictions is in the hospital.

On the other hand, the risk of toxic reactions is so reduced with local application in doses not exceeding 1 gm. (15 gr.) of the sulfonamides that this mode of usage appears reasonably safe, even among the ambulatory patients in dental practice.

TOPICAL USE OF SULFANILAMIDE

The efficacy of sulfanilamide depends on a minimum concentration of the free drug in the blood of about 8 mg. per 100 cc. Direct implantation of the pulverized drug, or instillation of an aqueous solution, in a region infected by vulnerable bacteria, procures the advantages of higher concentration and slower absorption from the strategic area, with reduced toxicity. This method has ample precedent and is being recommended with augmented favor by surgeons, urologists, (5, 6, 7, 8, 9) and others. Several dentists claim good results in the treatment of dry tooth-sockets by direct applications of sulfanilamide (10, 11, 12) but the reports are not detailed enough to permit experimental verification.

TREATMENT OF DRY-SOCKETS

In the first section of this survey, 17 cases of so-called dry socket were treated with sulfonamide compounds. Diagnosis was based on the concurrence of intractable pain and the absence of organized blood-clot in the socket. Cultures and smears were made routinely but were uninformative because contamination was evident in every case, and are therefore not noted.

The plan of treatment consisted of filling the sockets with powdered sulfonamide, securing its retention by sealing the orifice with a strip of gauze saturated with tr. benzoin. Dressings were repeated at least daily, in one instance (Case 5), four times in one day. Medication, including anodynes and mouth washes, was interdicted, but warm applications to the jaws were permitted at the discretion of the patient. Most patients were started with sulfanilamide, but in the later cases sulfapyridine or sulfathiazole were substituted during the course of treatment. Healing was generally preceded by the exfoliation of small, bone sequestra. Data are summarized in Table I. (Throughout the report the sulfonamide compounds are designated as follows: s-sulfanilamide, sp-sulfapyridine, st-sulfathiazole.)

No toxic reactions were observed. Published statistics indicating the period generally required for the resolution of necrotic sockets are not available, but my own records from 1930–1938, which include 61 cases treated symptomatically, show an average duration of 17.7 days. The extent to which this figure was reduced in 7 cases in this

series is impressive, and the average time of healing in this group as a whole was only 11.2 days. Coupled with the impression in many instances that there was amelioration in the *degree* of pain experienced, the favorable effect of chemotherapy becomes suggestive enough to warrant further experimentation. Accessory enteral doses of the sulfonamides, given in three cases did not appear sufficiently effective to warrant risking this form of therapy in dry-socket.

TABLE I

Dry-sockets treated with sulfonamide compounds

CASE NO.	TOOTH	DAYS POST-OPERATIVE BEFORE TREATMENT	NO. OF DRESSINGS WITH SULFONAMIDES			NO. OF DAYS TREATED	TOTAL ELAPSED DAYS FOR CURE
			S	SP	ST		
1	LR5	6	25			10	16
2	LL6	3	32			15	18
3	LL4	4	20			11	15
4	LL4	4	11			7	11
5	UR5	2	26			14	16
6	LR5	4	4			3	7
7	UR6	4	4	7		6	10
8	LL5	4	2	16		11	15
9	LR6	1	2	5		4	5
10	UL2	2	2	18		12	14
11	LL7	3	2	7		5	8
12	LR4	2		5		4	6
13	LR4	5	2	3	14	12	17
14	UL4	3		2	8	5	8
15[x]	LR6	4	4	14		11	15
16[y]	UL7	3	2	9		5	8
17[s]	LL5	1		2	12	12	13

[x] Additional therapy: Sulfanilamide, gr. 40 daily for 4 days.
[y] " " : Sulfapyridine, gr. 45 daily for 3 days.
[s] " " : Sulfathiazole, gr. 40 daily for 3 days.

MANAGEMENT OF IMPACTED THIRD MOLAR WOUNDS

In the second section of this survey, the effect of sulfonamide therapy in the control of postoperative pain was studied in a series of 81 extractions of partially or completely unerupted, impacted third molars. Experience with this type of operation indicated a high expectancy of moderate to severe reaction, consisting of pain, swelling, trismus, lymphadenitis and constitutional reaction. The first group

in this series consisted of 22 consecutive cases in which alternate patients were treated by the application of sulfonamides, leaving an equal number of controls. The findings are summarized in Table II

TABLE II

Third molar wounds treated with sulfonamides and controls

CASE NO.	TOOTH	SULFONAMIDE	ADDITIONAL DRESSINGS	P.O. TEMPERATURE (F)				DEGREE OF LOCAL REACTION				CLINICAL COMPLICATIONS
				1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	
1	LR8	S	2	99.3	99.6	99	99.4	B	B	A	A	
2	LL8		5	99.6	100.4	101	100.6	B	D	D	C	
3	LL8	S	2	99.8	98.8	99.4	99.6	B	B	B	A	Anesthesia of lip
4	UL8		4	101.6	101.3	99.8	100.3	D	C	D	D	Osteitic pain, 12 days
5	UR8	S	0	100.	98.8	99.2	?	A	A	B	?	
6	LL8		6	100.3	100	101.2	100.8	C	D	D	C	
7	LR8	S	2	100.6	100.6	98.8	99.	B	A	B	A	Secondary hemorrhage
8	UR8		2	98.6	99	99.2	99.	B	B	A	A	
9	LR8	S	1	99.4	99.2	98.8	?	B	B	A	?	
10	UL8		4	99.6	99.4	100.2	100.8	B	C	C	C	
11	UL8	S	1	98.2	99.2	99	99.1	B	C	B	B	Severe trismus, 3 days
12	UR8		6	100.4	99	100.8	100.5	C	C	D	B	
13	UR8	SP	2	99	99.6	100	99.2	B	A	B	A	Secondary hemorrhage
14	LL8		4	98.3	101	101.6	101.2	B	C	D	D	
15	UL8	SP	0	98.8	99.1	?	?	A	A	A	?	
16	UL8		3	100.4	101.2	100.	100.8	B	C	D	C	
17	LL8	SP	1	98.8	100.2	99.2	?	A	B	A	?	
18	LR8		5	100.2	100	100.8	101.2	C	D	C	D	
19	LL8	ST	2	100.2	100.8	99.2	99.4	B	B	A	B	Secondary hemorrhage
20	LL8		4	99.2	99.4	100.7	101	B	C	C	C	Severe pain, 10 days
21	LR8	ST	1	99.6	99.8	100	99.2	A	B	B	A	
22	UR8		3	99.8	99.6	100.6	100.8	B	B	C	C	

Summary: Average number of dressings: Treated group 2, Control group 5.

Average of local reactions: Treated group $\frac{A+B}{2}$, Control group C.

Average of maximum temperature: Treated group 99.6, Control group 100.9.

and *fig. 1.* The extent to which severe reactions were eliminated in patients receiving therapy was so arresting that experimental controls were dispensed with in the second group of 59 cases. The data

relating to this group appear in Table III, and a summary of all cases is made in Table IV.

Teeth were removed under procain anesthesia, employing the customary dissection technic, the sockets generously irrigated with warm saline solution and dried as completely as possible. At least 1 gm.

TABLE III

*59 third molar wounds treated with sulfonamides**

TEETH	S	SP	ST	MAXIMUM REACTION				MAXIMUM TEMPERATURE		
				A	B	C	D	Below 100°	Below 101°	Above 101°
Maxillary (33)	14			2	11	1	0	13	1	0
		12		3	9	0	0	11	1	0
			7	0	5	2	0	4	1	1
Mandibular (26)	14			1	12	1	0	13	3	0
		8		1	6	1	0	4	4	0
			4	0	2	1	1	1	1	2
Totals				7	45	6	1	45	11	3
Percentages				12	76	10	2	76	19	5

* Four patients in this group received general anesthesia: basal avertin followed by nitrous oxide and oxygen. The remainder received procain anesthesia.

TABLE IV

(Summary of Tables II and III)

Data in 70 treated and 11 untreated third molar wounds

	TOTAL	MAXIMUM REACTION				MAXIMUM TEMPERATURE		
		A	B	C	D	Below 100°	Below 101°	Above 101°
Sulfonamide Treated	70	8(11%)	54(77%)	7(10%)	1(1%)	52(74%)	13(19%)	5(7%)
Controls	11	0(0%)	1(9%)	3(27%)	7(64%)	1(9%)	4(36%)	6(55%)

(15 gr.) of powdered sulfonamide was firmly packed into the wound and its orifice covered with a light dressing of gauze saturated with tr. benzoin. The area was isolated from the saliva as completely as possible for several minutes to permit the benzoin solution to evaporate to a gummy consistency. Postoperatively, wounds were inspected each 24 hours for at least 4 days. With few exceptions, dressings were changed every second day and a sulfonamide applied

in the same manner as originally. Control cases were dressed with loose packings of gauze saturated in tr. benzoin.

Reactions in the form of pain, swelling and lymphadenitis were graded as absent (A), slight (B), moderate (C) and severe (D). Oral temperature was considered an index of constitutional reaction and was noted at each visit.

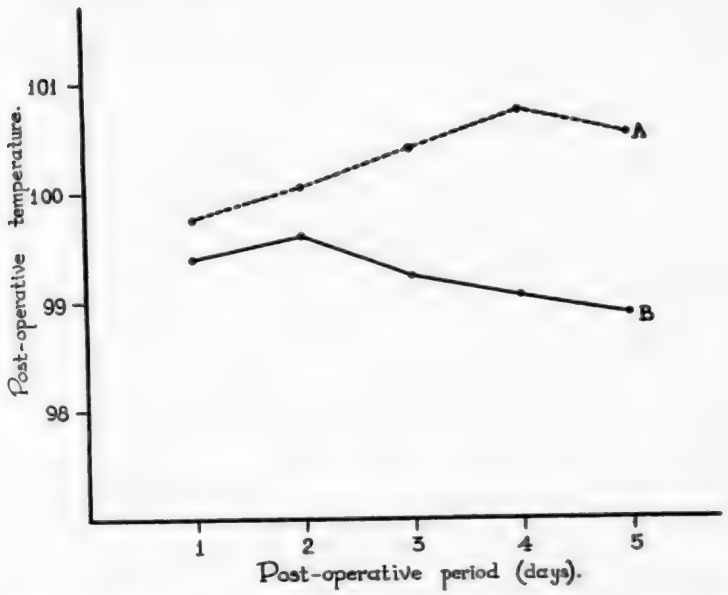

FIG. 1. Average postoperative temperature: 11 cases treated with sulfonamides and 11 control cases. (Wounds of impacted third molars). A = Sulfonamide treated. B = Control.

The most noteworthy effects of sulfonamide therapy appeared to be reduction of post-extraction pain, of constitutional reaction in the form of rise of temperature and reduction of the period requiring post-operative treatment. Less marked, yet unmistakeable was control of reactionary swelling and trismus. Although all sulfonamides appeared effective, sulfanilamide gave slightly superior results, followed in order by sulfapyridine and sulfathiazole. One disturbing observation was the relative frequency of secondary hemorrhage in the treated cases (13 per cent).

SIMPLE AND COMPLICATED EXTRACTIONS

The third section of this study deals with the effect of applying sulfonamides to the sockets of teeth in a series of 347 consecutive, ordinary extractions. The method was substantially the same as that followed in the previous experiments, excepting that the amount of pulverized drug used in each case was smaller. In most instances, the initial application appeared to be sufficient: in the cases exhibiting reactions of the C and D grades, supplementary dressings were applied as indications arose.

TABLE V

Sockets of extracted teeth treated with sulfonamides

REASON FOR EXTRACTION	SULFONAMIDE USED			DEGREE OF LOCAL REACTION				CLINICAL COMPLICATIONS
	S	SP	ST	A	B	C	D	
Periapical infection	84	63	46	101	83	8	1	Secondary hemorrhage, 6 cases
Periodontal infection	22	16	7	33	12	—	—	Secondary hemorrhage, 1 case
Imbedded roots	8	4	2	12	1	1	—	
Cyst	4	3	—	5	2	—	—	
Prosthetic strategy	12	10	7	15	13	1	—	
Pulp exposure	10	6	4	7	11	1	1	Dry socket, 1 case
Acute infection	21	16	2	18	19	2	—	Secondary hemorrhage, 1 case
Totals	161	118	68	191	141	13	2	
Percentages (approximate)				55	40	4	1	

Simple elevator and forceps technic was the method of choice; dissection through mucoperiosteum and alveolus was required in 63. Procain was the usual anesthetic agent, but for considered reasons, 18 patients received nitrous oxide and oxygen. A summary of the data including a classification of the indications for extractions is given in Table V.

As a control, similar data are recorded in Table VI for a consecutive series of 104 extractions of the same type. Procain anesthesia was used in all cases of the control group. In 26 cases, dressings of plain gauze were inserted; in the remainder, no dressings were used. *Fig. 2* graphically compares the degrees of reactions in percentages.

TABLE VI

Sockets of extracted teeth (treated routinely)

REASON FOR EXTRACTION	GAUZE DRESSING	NO DRESSING	DEGREE OF REACTION				CLINICAL COMPLICATIONS
			A	B	C	D	
Periapical infection	13	56	29	22	14	4	Secondary hemorrhage, 1 case
Periodontal infection		7	3	3	1	—	
Imbedded roots	1	3	—	2	1	1	Dry socket, 1 case
Prosthetic strategy		5	1	3	1	—	
Pulp exposure	2	6	1	2	3	2	Dry socket, 1 case
Acute infection	10	1	1	3	5	2	
Totals	26	78	35	35	25	9	
Percentages			33.7	33.7	24	8.6	

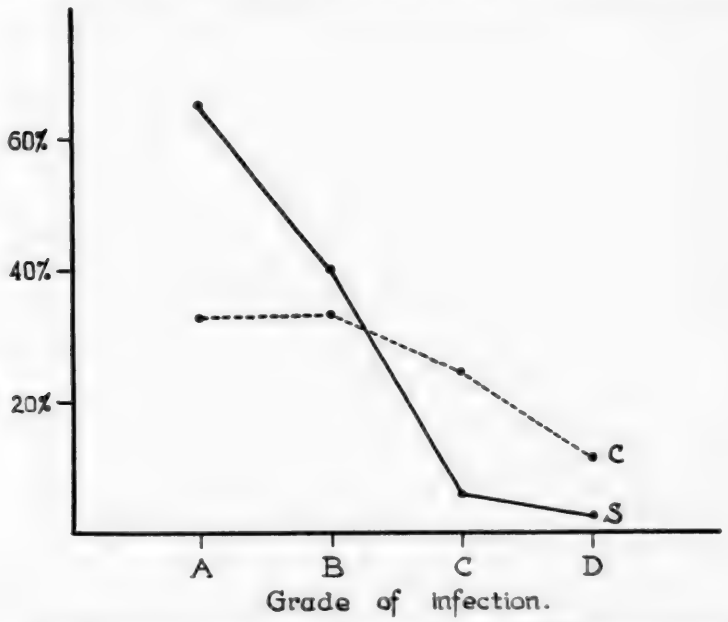

FIG. 2. Severity of local reactions in tooth-sockets. S = Sulfonamide treated. C = Control

The suppression of local reactions in the treated series in comparison to those in the control group, while not so striking as in the treated

group of third molar wounds, was unquestionable. This becomes most evident in examining the figures designating the severe reactions (C and D): 5 per cent for the treated group in contrast to 33 per cent for the untreated group.[2] Sulfanilamide again seemed to be the most effective drug in this series; sulfathiazole the least. The trend towards more secondary hemorrhages in the treated group (2.2 per cent) was diminished but persistent.

CONTAMINATED WOUNDS OF THE MAXILLAE

In the final section of this survey the effectiveness of the sulfonamides was studied in the treatment of wounds of root-resections, radicular and follicular dental cysts, of tooth-crowns surgically exposed for orthodontic treatment, and severely lacerated mucoperiosteum in compound fractures. Inasmuch as experience seemed to indicate a slight superiority of sulfanilamide, and because of its greater diffusibility, it was used in this series to the exclusion of the other drugs in the sulfonamide group. Dosage was proportioned to the size of the wound, varying from about 0.3 gm. (5 gr.) in the root-resection cases to 2 gm. (30 gr.) in one of the follicular cyst cavities. For purposes of these experiments, the wounds were completely closed by suture.

Exposed tooth-crown wounds were treated with the smaller dose of sulfanilamide and protected with a surgical paste having a zinc oxide-eugenol base. Open fracture wounds were treated with liberal applications of a creamy paste of sulfanilamide and water to the lesions three times daily. Complete immobilization of fragments was deferred for at least 7 days in each case to facilitate these applications.

Control data are not included in this group because of obvious variations in the nature of such cases as well as because of scarcity of material. Therefore, the observations on this series are presented as a note from which some impression, rather than conclusions, may be drawn.

[2] Attention is directed to the startling variance of these figures with those presented by Krashen (13) in his Figures I and II. A discrepancy of this degree cannot be satisfactorily accounted for by the fact that Krashen's series is much smaller. Unless the clue lies in unmeasurable intangibles, such as difference in clinical material or patient-resistance, the observations themselves, (and therefore the results of treatment) almost are diametrically opposite. Therefore the publication of a much similar material must be awaited in order to indicate which figures are valid.

Complete closure of wounds of this type by suture is often followed by considerable swelling and local discomfort. In but one case in this group however, that of a 3.5 x 2 x 2 cm. cyst involving the apices of the lower anterior teeth, was postoperative edema sufficiently severe to require removal of sutures before primary union had been completed.

Pain was inconspicuous in this series with 2 exceptions. The first was following the exposure of a maxillary canine crown in a 14 year old girl, in which severe periodontitis in the adjacent second incisor was experienced for a week. This appeared to be due purely to traumatic insult to the tooth, the root of which lay unusually close to the operative field. The second instance occurred in a 16 year

TABLE VII

Contaminated bone wounds treated with sulfanilamide

OPERATION	NUMBER	MAXIMUM LOCAL REACTION				PERCENTAGE OF MARKED REACTIONS
		A	B	C	D	
Root resections	14	9	5			0
Follicular cysts	2		2			0
Radicular cysts	4	1	2	1		25%
Exposed tooth-crowns	11	8	2	1		9%
Compound fractures	8	4	2	1	1	25%

old boy who suffered a fracture. A large submaxillary abscess developed and therefore this should be regarded as a distinct failure of chemotherapy.[3]

In general, however, it seems fair to record the impression that postoperative discomfort in this series was less than might, on the basis of past experiences, reasonably be expected.

GENERAL DISCUSSION

In the light of these observations, chemotherapy exerts a remarkable clinical effect in operative wounds of the jaws. With few exceptions among nearly 500 unselected patients, topical application of the sulfonamides moderated postoperative discomfort, irrespective of the degree of traumatism involved by the procedure. As the predominant

[3] This case-history and its subsequent treatment by chemotherapy will be presented in detail in a subsequent paper.

effect of these drugs is bacteriostatic, their influence in these cases may be presumed to be chiefly a neutralization of contamination. If these results are consistently duplicated, infection, rather than tissue damage, will have to be considered the essential factor in producing inflammatory reactions following extraction. Traumatism becomes secondary, although its avoidance is still imperative because the more extensive tissue damage becomes, the wider are the avenues opened for the invasion of contaminated material.

Once a marked pathologic alteration of the tissues occurs, as in necrotic tooth-sockets, control by chemotherapy appears to be less effective, although probably not eliminated. The local processes of tissue repair may be accelerated, or intensified, but they follow the same pattern and arrive at the same termination as if unmodified by chemotherapy. For example, liquifying tissues go on to suppuration and necrotic bone sequestrates.

In topical use, the relative efficiency of the sulfonamides seems to depend on their solubility and diffusibility quite as much as on a specificity for certain organisms. The complete safety of this method is indicated by the total absence of clinically observable toxic drug reactions in a statistically important number of patients. It is true that the incidence of secondary hemorrhage is notable, but scarcely to an extent to be alarming. The question of whether chemotherapy predisposes to this complication however is of interest and should be checked by careful future notations.

SUMMARY

Under favorable conditions, the topical application of the sulfonamides seems to reduce the pain, and to a more limited degree, other untoward post-operative reactions of minor surgical wounds of the maxilla and mandible. This inference cannot be accepted as a valid conclusion until it is consistently corroborated. Publication of observations on similar studies is desirable.

The apparent safety of this method, its simplicity, and the necessity for defining the scope of chemotherapy in oral surgery invites wide experimentation.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

SCIENTIFIC PROCEEDINGS OF THE NEW YORK SECTION
JANUARY 28, 1941[1]

Compiled by FRANCES KRASNOW, PH.D., Editor, New York Section

1. PRELIMINARY REPORT OF LOCAL MICRODETERMINATIONS OF GLUCOSE. *Samuel C. Miller, D.D.S.*, and *Benjamin B. Seidler, A.B., D.D.S.*,* College of Dentistry, New York University, New York, N. Y. In a previous study, it was found that in precocious, advanced alveolar bone destruction there was consistently a flattened glucose tolerance curve. This study was undertaken in an attempt to ascertain the relation between the alveolar disintegration and the glucose curve. The method utilized was to compare the glucose concentration in the gingival capillary blood with that of the finger capillary blood. A modification of Benedict's microglucose technique was employed. Patients were selected at random from the dental clinic of New York University. The clinical appearance of the gingival tissue was noted and radiograms of the mouth were obtained. An interproximal papilla was incised with a sharp scalpel and 50 ml. of blood were pipetted into a tube containing 2.5 ml. of dilute precipitating solution. This was repeated from a puncture of the palmar surface of the finger. There were no significant differences obtained when the normal or control cases were compared with cases of alveolar destruction, or, when bone types were compared.

2. THE PRODUCTION OF ACIDS FROM GLUCOSE BY ORAL MICRO-ORGANISMS. *William H. Summerson, Ph.D.*,* and *Isaac Neuwirth, Ph.D.*, Department of Biochemistry, Cornell University Medical College and Department of Pharmacology and Therapeutics, College

[1] The cost of publication of these sectional proceedings is paid by the New York Section.
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* Guest.

of Dentistry, New York University. This is a study by chemical and micromanometric methods of the production of acid from glucose by oral microörganisms in saliva as a medium. The saliva containing the mixed oral microörganisms was obtained by paraffin stimulation from subjects with dental condition varying from severe involvement to absence of caries. The experiments were carried out by incubation with added glucose in the Warburg apparatus at 37° for 15 or 30 minute periods, using a differential manometer for the precise determination of the total acid produced, and improved chemical methods for the subsequent analytical study of the individual acids present. It was found in all cases studied that lactic acid production accounted for only 50 per cent or less of the total acid produced, with no significant differences in this respect between carious and non-carious individuals. Pyruvic acid was occasionally found in small amounts, but lactic and pyruvic acid together in no way account for the total acid produced. The nature of the extra acid or acids is under investigation.

3. A COMPARISON OF DENTAL CARIES ON THE BUCCAL AND PROXIMAL SURFACES OF PREMOLAR TEETH. *James M. Dunning, D.D.S.*, Metropolitan Life Insurance Company, New York, N. Y. To compare the incidence by age of cervical with other types of caries, Hyatt's figures for 12,645 employees, between the ages of 17 and 60, of the Metropolitan Life Insurance Co., New York, were re-analyzed. Premolar teeth were selected for the study of proximal and cervical lesions because the likelihood of buccal caries in any other position than cervical was almost eliminated. Combinations of buccal and proximal lesions with lesions on other surfaces were added to the buccal and proximal totals. Because of possible errors, no attempt was made to evaluate occlusal or recurrent caries. Results were expressed in lesions per 100 tooth surfaces, i.e., the percent proportion of the buccal or proximal affected surfaces to the number of buccal or proximal surfaces available for localized caries (surfaces remaining after those represented by missing, crowned, or broken-down teeth had been deducted from total possible surfaces). Proximal caries was found to rise rapidly to a high point between the ages of 30 and 34 years,

after which it remained essentially level. Buccal (or, by inference, cervical) caries rose evenly throughout the entire period of the study.

4. THE INCIDENCE OF ROOT RESORPTION OF VITAL PERMANENT TEETH. *Samuel Hemley, D.D.S.*, Department of Orthodontia, College of Dentistry, New York University. The incidence of root resorption of vital permanent teeth has become a matter of increasing concern to orthodontists ever since attention was first directed to its occurrence by Ketcham in 1929. Recent reports by Rudolph showing the high incidence of resorption of roots in a series of orthodontic patients justifies alarm. In fact, his findings prompted a similar check in the Department of Orthodontics at the College of Dentistry, New York University. Where heretofore the percentage of the incidence was determined entirely by the number of cases in which teeth were involved, in this study, the individual teeth were carefully analyzed, both as to the degree of root resorption which developed and the percentage of teeth involved. This study was made on a series of 195 cases. The findings show conclusively that the incidence of root resorption is very much less than has been supposed and that only rarely does root resorption develop to a degree to be of much concern. (Published in full, *J. D. Res.* **20:** 133, 1941.)

5. THE DEVELOPMENT OF FIBERS IN HUMAN DENTIN. *Gerrit Bevelander, Ph.D.*, Department of Anatomy, College of Dentistry, New York University. The origin and structure of the fiber system of human dentin was studied in several successive developmental stages of the tooth. Sections were stained with silver and examined by means of polarized light. This study shows (1) reticular fibers derived from the pulp become oriented in a radial direction, (2) these fibers extend from the pulp to the dentino-enamel junction and in the latter region the fibers are coarse and extremely argyrophylic, (3) as calcification proceeds, some of the fibers undergo a rearrangement appearing at eruption in a transverse or tangential direction. Observations made on dentin of mature specimens show that the peripheral zone retains its embryonic characteristics. The fibers in the pulpal zone retain an arrangement similar to that observed in the

new born. They are, however, much more extensive and exhibit very little affinity for silver.

6. CONCERNING THE "VITALITY" OF THE CALCIFIED DENTAL TISSUES: THE VITAL STAINING OF THE INNER ZONE OF HUMAN DENTAL ENAMEL. *Charles F. Bodecker, D.D.S.*, and *William Lefkowitz, D.D.S.*, Laboratory of Oral Histology, School of Dental and Oral Surgery, Columbia University, New York, N. Y. Abstract to be published with proceedings of International Association for Dental Research (*J. D. Res.* **20,** June 1941).